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Thermal Tolerance of Aquatic Insects Inhabiting the Tennessee River-Reservoir System

Tennessee Valley Authority, Muscle Shoals, AL

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In laboratory tests, nymphs of Hexagenia bilineata (Ephemeroptera) and larvae of Chironomus crassicaudatus (Diptera) were highly tolerant of short term thermal shocks (six hour duration, simulating entrainment in a thermal plume and drifting to ambient). TL50 values increased from 35°C at an acclimation temperature of 5°C to 38-40°C at an acclimation temperature of 20-25°C. However, survivors of these treatments experienced higher percentages of delayed mortality compared to controls after being held for ten days to four weeks at the original acclimation temperature. The most sensitive stage found in the life cycle of H. bilineata was the egg during ovipoisition (time of fertilization); eggs exposed to 33°C for 15 minutes during oviposition hatched at a significantly lower percentage than controls (ambient was 28°C).

Field data generated indicated that aquatic insect species inhabiting TVA's large warm-water reservoirs are living close to their thermal maximum, and that an upper limit of 33°C would ensure the maintenance of sizeable populations of the species studied.

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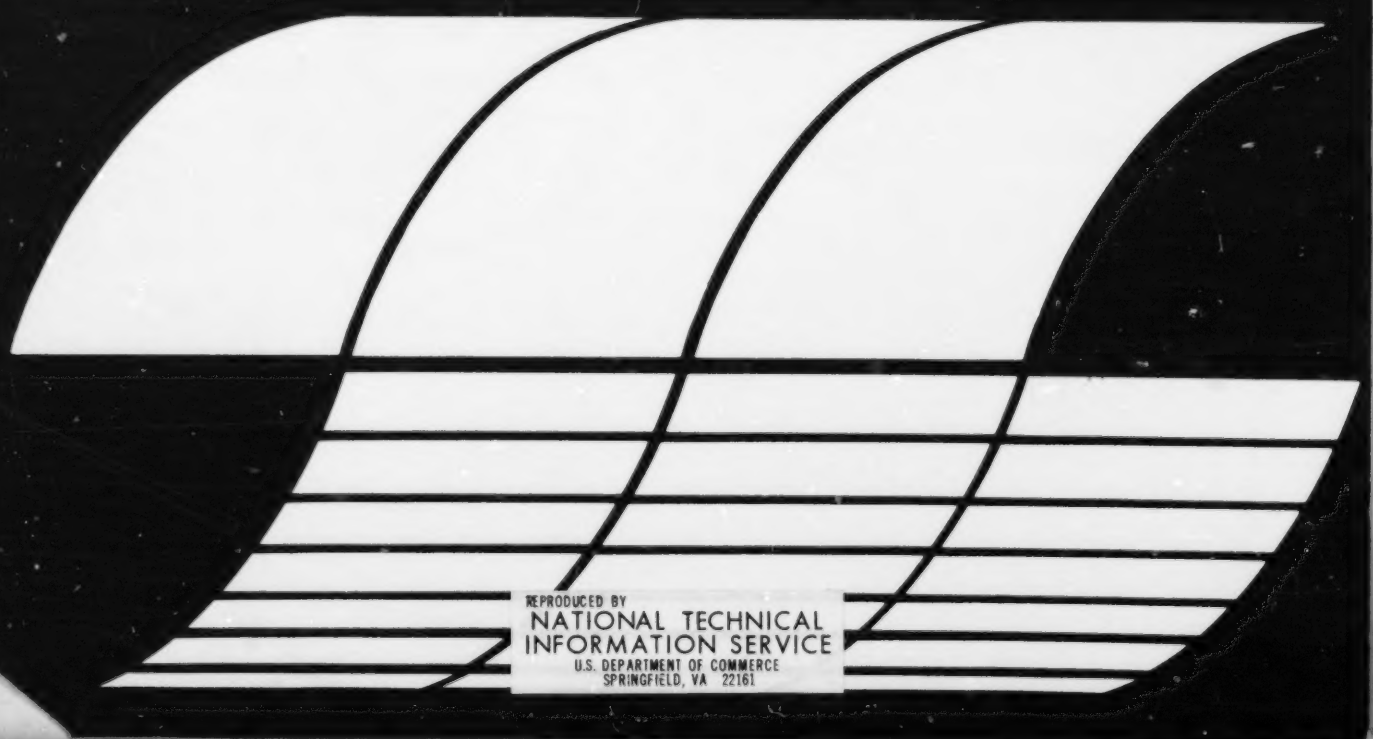

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THERMAL TOLERANCE OF AQUATIC INSECTS INHABITING THE TENNESSEE RIVER-RESERVOIR SYSTEM

by

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CONTENTS

	Page
Abstract	iv
List of Figures	v
List of Tables	viii
List of Abbreviations and Symbols	ix
Acknowledgements	x
1. Introduction	1
2. Conclusions	2
3. Recommendations	4
4. Experimental Procedures	5
Laboratory Experiments	5
Field Studies	7
5. Results and Discussion	13
Thermal Tolerance of Immature Stages	13
Thermal Tolerance of Egg Stage	21
Population Structure of *Hexagenia bilineata* in Thermal Plume Areas	31
References	62
Glossary	65

ABSTRACT

The objectives of this research project were to (1) determine the short-term thermal tolerance of several important species of aquatic insects in the Tennessee River - Reservoir system, (2) investigate the effect of acclimation temperature on tolerance, and the possibility of delayed mortality, (3) determine the most sensitive stage in the life cycle, and (4) conduct field studies on growth and emergence of selected aquatic insects within actual thermal plume areas.

In laboratory tests, nymphs of Hexagenia bilineata (Ephemeroptera) and larvae of Chironomus crassicaudatus (Diptera) were highly tolerant of short term thermal shocks (six hour duration, simulating entrainment in a thermal plume and drifting to ambient). TL50 values increased from 35°C at an acclimation temperature of 5°C to 38-40°C at an acclimation temperature of 20-25°C. However, survivors of these treatments experienced higher percentages of delayed mortality compared to controls after being held for ten days to four weeks at the original acclimation temperature. The most sensitive stage found in the life cycle of H. bilineata was the egg during oviposition (time of fertilization); eggs exposed to 33°C for 15 minutes during oviposition hatched at a significantly lower percentage than controls (ambient was 28°C).

Density of Hexagenia bilineata nymphs varied between stations upstream and downstream of three TVA electric-generating plants and between sampling dates, although overall populations in thermal plume areas were comparable to those in ambient areas. Nymphs of H. bilineata collected at stations within the thermal discharge areas were larger on the average than nymphs from ambient stations prior to the first emergence of the year. Larger percentages of nymphs ready to emerge at the thermal stations also indicated accelerated development; however, the first emergence occurred simultaneously at heated and ambient stations at the plant observed.

The data generated indicate that aquatic insect species inhabiting TVA's large warm-water reservoirs are living close to their thermal maximum, and that an upper limit of 33°C would ensure the maintenance of sizeable populations of the species studied.

This report was submitted by the Tennessee Valley Authority, Division of Water Resources, in partial fulfillment of Energy Accomplishment Plan 80-BDR under terms of Interagency Agreement D8-E721-DR with the Environmental Protection Agency. Work was completed in March 1981.

FIGURES

Number		Page
1	Outline map of Kentucky Lake in vicinity of Johnsonville Steam Plant showing locations of the five sampling stations	10
2	Outline map of Old Hickory Reservoir in vicinity of Gallatin Steam Plant showing locations of the five sampling stations	11
3	Outline map of Wheeler Reservoir in vicinity of Browns Ferry Nuclear Plant showing locations of the four sampling stations	12
4	Mortality responses of larvae of Chironomus crassicaudatus to 6-hour thermal shocks after acclimation at three temperatures (5, 15, and20°C)	16
5	Temperature tolerance triangle for larvae of Chironomus crassicaudatus from Wheeler Reservoir (TRM 298), Limestone County, Alabama; based on 6-hr exposure to elevated temperature	17
6	Mortality responses of Hexagenia bilineata nymphs subjected to 6-hour temperature shocks after acclimation to 5, 15, and 25°C	18
7	Temperature tolerance triangle for Hexagenia bilineata from Spring Creek embayment, TRM 283.0, Lawrence County, Alabama, based on 6-hour exposures to elevated temperatures	18
8	Mean percentages of hatching of Hexagenia bilineata eggs in response to main effects of shock temperature, shock duration, and hatching day	24
9	Effect of shock temperature and shock duration on the mean cumulative percentage of hatching of Hexagenia bilineata eggs	27
10	Survival of Hexagenia bilineata nymphs 4 weeks after exposure to short duration thermal shocks in the egg stage	27

FIGURES (continued)

Number		Page
11	Vertical temperature profiles at five stations in vicinity of Johnsonville Steam Plant, Kentucky Lake, from February to November 1978	39
12	Vertical temperature profiles at five stations in vicinity of Gallatin Steam Plant, Cumberland River, from February to September 1978	41
13	Vertical temperature profiles at five stations in vicinity of Browns Ferry Nuclear Plant, Tennessee River, from February to September 1978	43
14	Bottom temperatures at two stations near Johnsonville Steam Plant, Gallatin Steam Plant, and Browns Ferry Nuclear Plant from February to December 1978	45
15	Comparison of differences in river bottom WT's at the thermal plume stations where H. bilineata nymphs were collected in the vicinity of Johnsonville and Gallatin Steam Plants and Browns Ferry Nuclear Plant	46
16	Density of Hexagenia bilineata nymphs at stations 1 and 3 near Johnsonville Steam Plant from March to October 1978 based on 10 Ponar samples per station per month	47
17	Density of H. bilineata nymphs at stations 1 and 4 near Gallatin Steam Plant from March to October 1978; based on 10 Ponar samples per station per month	48
18	Density of H. bilineata nymphs at stations 1 and 3 near Browns Ferry Nuclear Plant from March to September 1978; based on 10 Ponar samples per station per month	49
19	Mean head width of Hexagenia bilineata nymphs collected at stations 1 and 3 near Johnsonville Steam Plant from March to October 1978	50
20	Mean head width of H. bilineata nymphs collected at stations 1 and 4 near Gallatin Steam Plant from March to September 1978	51
21	Mean head widths of H. bilineata nymphs collected at stations 1 and 3 near Browns Ferry Nuclear Plant from March to September 1978	52

FIGURES (Continued)

Number		Page
22	Comparisons of frequench of Hexagenia bilineata nymphs in 0.2 mm size classes of head width at stations 1 (ambient) and 3 (thermal near Johnsonville Steam Plant.	53
23	Comparisons of frequency of Hexagenia bilineata nymphs in 0.2 mm size classes of head width at stations 1 (ambient) and 4 (thermal) near Gallatin Steam Plant	56
24	Comparisons of frequency of Hexagenia bilineata nymphs in 0.2 mm size classes of head width at stations 1 (ambient) and 3 (thermal) near Browns Ferry Nuclear Plant	59

TABLES

Number		Page
1	Direct and delayed mortality of C. crassicaudatus larvae acclimated to 5, 15, and 20°C and exposed to 6-hr thermal shocks.	14
2	Results of thermal shock tests on nymphs of H. bilineata acclimated to 5, 15, and 25°C	19
3	Percentages of mortality of first-instar nymphs of H. bilineata acclimated to 25°C and exposed to 6-hr thermal shocks . . .	20
4	Percentages of hatching of H. bilineata eggs exposed to thermal shocks for three durations eight hours after oviposition. .	22
5	Analysis of variance of three-factor thermal experiment on eggs of Hexagenia bilineata	25
6	Analysis of variance and partitioning of two main treatments, shock temperature and shock duration on hatching success of H. bilineata eggs on the fourth day of hatching	26
7	Hatching success and survival of hatchlings of argia moesta exposed to five constant temperatures during egg stage. . .	29
8	Hatching success and survival of hatchlings of argia translata exposed to five constant temperatures during egg stage. . .	30
9	Composition of particle sizes (%), within sediment samples taken in April 1978 from selected stations at Johnsonville Steam Plant, Gallatin Steam Plant, and Browns Ferry Nuclear Plant	32
10	Results of head width measurements of hexagenia bilineata nymphs collected at thermal and ambient stations near Johnsonville and Gallatin Steam Plants and Browns Ferry Nuclear Plant. .	34
11	Number of H. bilineata nymphs posessing darkened wing pads at the ambient and thermal stations near Johnsonville and Gallatin Steam Plants and Browns Ferry Nuclear Plant. . .	36

LIST OF ABBREVIATIONS AND SYMBOLS

ΔT -- change in temperature above ambient
^{o}C -- degrees Celsius
df -- degrees of freedom
DO -- dissolved oxygen
hr -- hour
km -- kilometer
m -- meter
min -- minute
ml -- milliliter
mm -- millimeter
n -- number of individuals in a sample
P -- probability
r -- correlation coefficient
s -- standard deviation
Tacc -- acclimation temperature
TRM -- Tennessee River mile
TVA -- Tennessee Valley Authority
$\bar{x}$ -- mean value

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SECTION 1

INTRODUCTION

The discharge of condenser cooling water from electric-generating stations raised concern in the 1960's about the maintenance of a diverse, indigenous flora and fauna in receiving bodies of water. As the demand for electric power has increased, so has the amount of heated water discharged to rivers, reservoirs, and lakes, causing further alteration of temperature regimes in these environments. Temperature has long been known to be a major factor influencing the development and physiological well being of organisms. However, specific knowledge of the tolerance levels of a variety of organisms throughout their life cycles is needed in order to establish guidelines to protect aquatic ecosystems.

An integral part of the food web and energy transfer within freshwater ecosystems is the aquatic insect fauna. Many fish rely on insects for a large portion of their diet (Howell et al. 1941, Larimore 1957, Applegate and Mullan 1967, Siefert 1969, Timmons, et al. 1981). Although the effects of temperature have been studied in several groups of aquatic insects, relatively little detailed information is available on the thermal sensitivity of benthic insects in the southeastern United States. The purpose of this study is to provide data on the thermal tolerances of selected, dominant species of aquatic insects in the Tennessee River-Reservoir system.

This report is a synthesis of results obtained from a variety of experimental and field studies conducted by TVA during a 5-year period (1975-1980). These studies were directed at (1) establishing thermal tolerance of various immature stages of aquatic insects, (2) determining the most sensitive stage in the life cycle, (3) investigating effects of temperature changes on reproduction, growth, and development, (4) analyzing selected species in the field in regard to population size and structure, growth, and emergence (areas in heated discharge compared to control areas), and (5) providing recommendations on guidelines for thermal discharges based on this and other published reports.

SECTION 2

CONCLUSIONS

Immature stages of aquatic insect species tested (Hexagenia, Chironomus, Argia) are tolerant of relatively high temperature increases, as high as 35-40°C, based on TL50 values determined by short-term laboratory experiments. The findings agree in general with other thermal requirement studies on insects that inhabit large, warm-water rivers and reservoirs. Acclimation temperature was found to affect tolerance, indicating that there are seasonal effects on tolerance. Immature insects surviving thermal shock for brief periods (up to six hours), such as drifting into a thermal plume, may experience a delayed mortality, depending on acclimation and thermal plume temperatures. Very small nymphs (first instars) of Hexagenia are less tolerant of thermal shock than are larger nymphs (>15 mm long), indicating that the entire life cycle must be considered in establishing upper-thermal requirements of a species. The sharp response from a very low percentage of mortality to a high percentage with an increase of shock temperature of only 3°C indicates that immature aquatic insects are living very near their thermal maximum.

Eggs of H. bilineata develop and hatch successfully (over 90 percent) at constant temperatures of up to 34°C, and can withstand very brief (5-15 min) exposures to temperatures as high as 41°C after fertilization. However, if eggs are laid by females directly into heated water (fertilization takes place during oviposition), a significant decrease in hatching occurs at temperatures as low as 33°C. Therefore, the fertilization process appears to be the most sensitive stage in the life cycle of this species. Delayed mortality was also found in nymphs exposed to brief (5-15 min) thermal shock in the egg stage. Eggs of Argia, which are laid near the water's surface, are at least as tolerant of high constant temperature as those of Hexagenia.

Based on field studies, growth of Hexagenia nymphs appears to be accelerated in areas of warm-water discharge from several of TVA's electric-generating plants. Also, size appears to be increased, although such comparisons are inadequate for delineating thermal effects because the population is comprised of differently aged individuals, and other variables could account for the differences. No advanced emergence of adults in these areas was observed.

Results of this study indicate that the most sensitive stage of the dominant aquatic insects of the Tennessee River-Reservoir system would be protected if water temperatures did not exceed 33°C in summer. Thermal discharges of high ΔTs from TVA's electric-generating plants are usually very limited in area affected, and cooling is rapid even when river temperatures reach 30°C naturally in summer. Upper limits for other seasons can not be determined from our data. Although short-term TL50 values indicate tolerance to temperatures up to 30°C when acclimated to 5°C, long-term studies on latent and chronic effects would be required for further assessment.

SECTION 3

RECOMMENDATIONS

Thermal discharge guidelines should be based on local, seasonal conditions and the most sensitive stage in the life cycles of those species to be protected. For the large, warm-water reservoirs of the Tennessee River examined in this study, an upper limit of 33°C in summer should protect the dominant species of aquatic insects.

Discharges with ΔT's below 9°C during colder times of the year should protect against acute mortality. However, long-term studies on fecundity and development would be required to establish definite protective limits during cold seasons, as chronic effects and advanced adult emergence can be expected even at low ΔT's.

SECTION 4

EXPERIMENTAL PROCEDURES

BACKGROUND

Several criteria were used to select two representative species, a burrowing mayfly, Hexagenia bilineata (Say) (Ephemeroptera: Ephemeridae), and a midge, Chironomus crassicaudatus (Malloch) (Diptera: Chironomidae) for use in this study. Large populations of these eurythermal species inhabit the Tennessee River reservoirs, facilitating experimentation and data gathering. Evaluation of thermal effects on these species is meaningful because they occur in areas of power plant thermal discharge. The two species represent two different types of insect life histories, mayflies being hemimetabolous whereas midges are holometabolous. In addition, these insects are important food items for fish, and can be maintained fairly easily in the laboratory. Several other species, including damselflies in the genus Argia, were used for comparative data.

LABORATORY EXPERIMENTS

Experiments were designed to determine TL50 values (temperature at which 50 percent mortality occurs) for immature stages held at various acclimation temperatures, and to determine tolerance of the egg stage.

Thermal Tolerance Tests On Immature Stages

Larvae of Chironomus crassicaudatus and nymphs of Hexagenia bilineata were collected from overbank sediments in Wheeler Reservoir at Spring Creek embayment (TRM 283) and the mouth of Round Island Creek (TRM 298) at times when substrate temperatures approximated desired acclimation temperatures. Prior to testing, the insects were held for one week in aerated aquaria containing 4 to 5 cm of mud. Nymphs of H. bilineata larger than 15 mm in length and fourth instar larvae of C. crassicaudatus were used for testing. Nymphs of H. bilineata were acclimated to 5, 15, and 25°C, whereas larvae of C. crassicaudatus were acclimated to 5, 15, and 20°C (no larvae were found at time of year when bottom temperature was 25°C or higher).

On test days, insects were gently removed from the mud and placed directly into water maintained at a range of ΔT's. Test water was continuously aerated to maintain dissolved oxygen near saturation

level. The ΔT interval was 3°; the maximum ΔT's ranged from 18 to 34°, depending on acclimation temperature. Tests were replicated on separate days; one replicate consisted of 10 to 30 individuals per ΔT. An exposure duration of 6 hr was chosen to approximate the maximum amount of time drifting individuals may be exposed to thermal plumes. Furthermore, these species swim vigorously in open water when no substrate is provided for burrowing; longer exposure times result in fatigue which could affect survival.

Following exposure for 6 hr, mortality was recorded and survivors were slowly brought back to acclimation temperature (1°/hr) and placed in aquaria with mud substrate. Two to four weeks later delayed mortality was recorded. TL50 values for the 6 hr and longer-term tests were calculated separately from regression equations by giving the dependent variable (mortality) a value of 50 percent. An R^2 value was computed to measure the proportion of variation about the mean percentage of mortality that is explained by each linear regression equation (see Draper and Smith 1966, p. 26). Temperature tolerance triangles were constructed following McErlean, et al. (1969).

Thermal Tolerance Studies On Eggs

Eggs of H. bilineata were collected from gravid females on June 19, 1979, at Pickwick Reservoir (TRM 245) near Colbert Steam Plant, Lauderdale County, Alabama. Water temperature at time of collection (2100 CDT, beginning of oviposition) was 26°C. The eggs were transported to the laboratory, and eight hours later were stirred and transferred in equal proportions to four large glass dishes containing dechlorinated tap water at four different temperatures--26 (ambient), 31, 36, and 41°C, yielding ΔT's of 0 (control), 5, 10, and 15°C. Eggs were transferred to ambient after exposure for 5, 10, and 15 min, to give 12 temperature-time treatments. For each treatment, twelve replicate flow-through containers (see Tennessen and Miller 1978) were set up, each containing approximately 1000-1500 eggs. The containers were placed in aerated aquaria held at 26°C in refrigerator-size incubators.

When hatching began, three containers per treatment were removed each day for four days, and the number of eggs hatched in a subsample of 300 eggs was counted. These counts were transformed to percentage hatched and were analyzed by an analysis of variance and Sheffe's multiple comparison test (Li 1964, p. 273).

As a preliminary check on survival of young nymphs hatching from these treatments, 100 nymphs that hatched on June 30, 1979 from the 15 minute exposures to each ΔT were kept in aerated aquariums containing 3 cm of mud. One month later (July 29) surviving nymphs were recovered and counted. Because only one replicate per treatment was used, no statistical analysis was performed.

Mature females of the damselflies Argia moesta (Hagen) and A. translata Hagen were collected along the Tennessee River at TRM 254.5. They were transported to the laboratory and put in screened cages containing bibulous paper rolled into "paper logs" in 1 cm of water. The next day, "logs" in which eggs had been deposited were removed and incubated at five temperatures: 28° (ambient), 31°, 34°, 37°, and 40°C (daily fluctuation of ± 0.5, except ambient which fluctuated ± 2°C). Photoperiod was 6 a.m. to 7 p.m. (13L:11D). The dishes containing the floating logs were constantly aerated. Because most females would not lay eggs under these laboratory conditions, we were unable to gather the number of egg-laden logs (replicates) required by the statistical design. Therefore, results are merely tabulated and relative comparisons are made.

When hatching commenced, the following data were recorded over a 4-day period (A. moesta) or 9-day period (A. translata) for each temperature: (1) number of nymphs hatched, (2) total number of eggs exposed, and (3) percentage of hatching. The nymphs were held at ambient temperature and fed zooplankton for one week, after which the number surviving was recorded.

FIELD STUDIES

The TVA electric-generating stations chosen for field studies were Johnsonville Steam Plant, located in Humphreys County, Tennessee, Gallatin Steam Plant, located in Sumner County, Tennessee, and Browns Ferry Nuclear Plant, located in Limestone County, Alabama.

Description of Study Areas

Johnsonville Steam Plant is comprised of ten units with a total generating capacity of 1,485 MW. It is situated on Kentucky Reservoir of the Tennessee River (TRM 99.5). The reservoir is approximately 2.2 km wide in this area and is composed of two regions: the main channel, about 300 m wide nearest the plant, and an extensive, shallow overbank (Figure 1). The condenser cooling water system of the plant has a maximum capacity of 65 m^3/s, which is discharged into a 308 m by 1,128 m basin that empties into the reservoir.

Five sampling stations were selected for temperature recordings. Mayflies were collected at station 1, about 1.5 km upstream from the plant intake, and at station 3, within the influence of the thermal discharge.

Gallatin Steam Plant is situated on Old Hickory Reservoir of the Cumberland River between CuRM 242.0 and 244.0. This fossil-fueled steam plant is comprised of four units with a total generating capacity of 1,255 MW. The condenser cooling water system of the plant has a maximum capacity of 40.1 m^3/s which is discharged into a 1,005.8 m long canal which empties into Old Hickory Reservoir. The five sampling stations were located as shown in Figure 2. Mayflies were collected at stations 1 (control) and 4 (thermal).

Browns Ferry Nuclear Plant is comprised of three separate units with a total generating capacity of 3,456 MW. It is situated on the north shore of Wheeler Reservoir of the Tennessee River (TRM 294). The condenser cooling water system of the plant requires a maximum of 123 m^3/s, which may be discharged into diffusers that distribute the condenser water across the channel or divert it to the mechanical draft cooling towers. Four sampling stations were selected (Figure 3), and mayflies were collected at stations 1 (control) and 3 (thermal).

Recording Vertical Temperature Profiles

Vertical temperature profiles were recorded at the sampling stations upstream (ambient) and downstream (thermal) of the three generating plants. Temperatures were recorded monthly with a calibrated thermistor from surface to river bottom at 1-m intervals from February to December 1978. Temperature at river bottom was used to indicate when the thermal plume reached the benthic habitat and to compare the magnitude of differences in substrate temperature between stations.

Sediment Analyses

Sediment samples were collected April 20-27, 1978, to check for uniformity of substrate at stations sampled for H. bilineata nymphs. Samples were placed in plastic bags and kept cool without preservative.

In the laboratory the wet samples were split with a Jones sample splitter until a small, random subsample was obtained (20-40 g). The subsample was sieved through a 200-mesh screen; the residue left on this screen was dried and weighed. The material passing through the sieve was dispersed in 100 ml of Calgon® solution and allowed to settle in a 1,000-ml graduated cylinder filled with distilled water for 24 h prior to standard pipette analysis. At prescribed time intervals at predetermined depths, 20-ml aliquots were taken, dried, and weighed. The percentage composition of phi sizes 4-10 (Krumbein scale) was determined from these dry weights.

Volatile solids were determined by ashing a sample (100-200 gms) for 12 hours at 600°C. Samples were weighed, placed in the furnace for 12 hours and then cooled in a desiccator. After cooling the samples were reweighed to determine the mineralization loss.

Collecting and Measuring Hexagenia Nymphs

Ten Ponar grab samples were taken at the ambient station and at one of the thermal plume stations at each plant monthly from March to October 1978. The samples were washed in the field on a 48-mesh sieve and the mayfly nymphs were preserved in 80 percent ethanol. To compare size of the nymphs, head capsule widths were measured with an ocular micrometer on a binocular microscope. Size categories of 0.2 mm intervals were established and the numbers of nymphs in these categories were transformed to percentages. Histograms were used to compare population

structure at the two stations for each month. Mean head width and the percentage of nymphs having blackened wing pads (indication of maturity and readiness to emerge to adult stage) were also calculated.

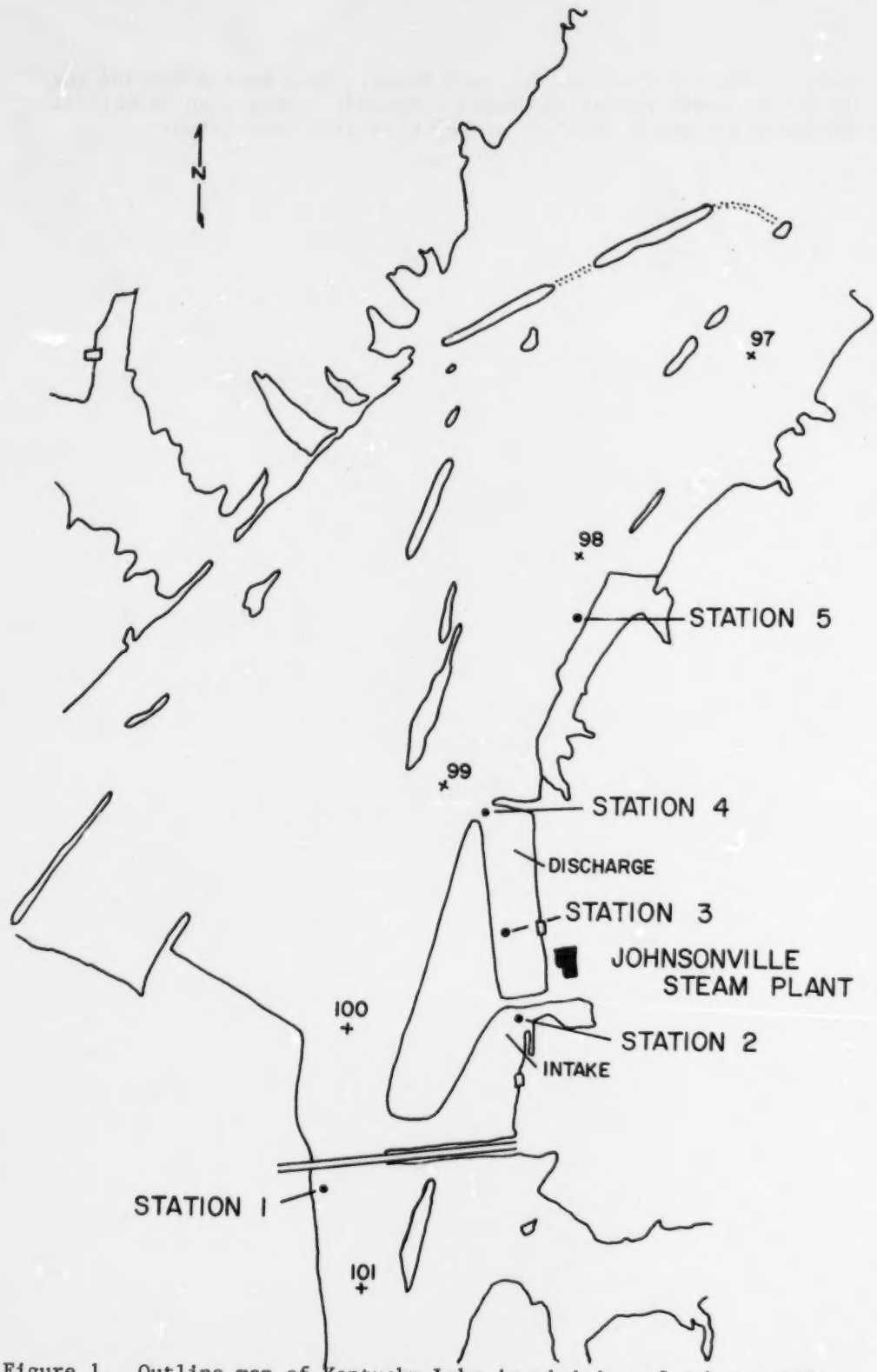


Figure 1. Outline map of Kentucky Lake in vicinity of Johnsonville Steam Plant showing locations of the five sampling stations.

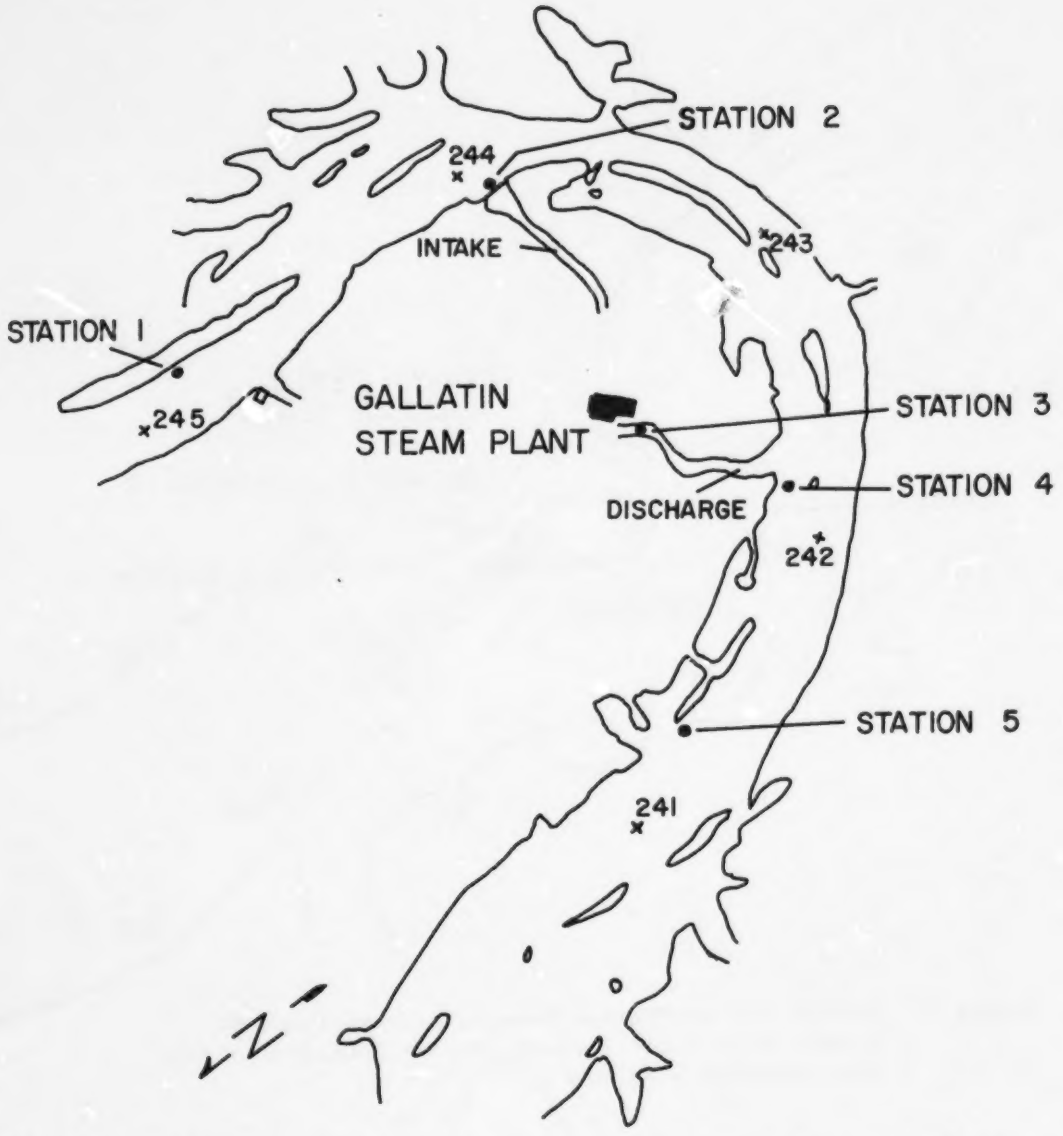


Figure 2. Outline map of Old Hickory Reservoir in vicinity of Gallatin Steam Plant showing locations of the five sampling stations.

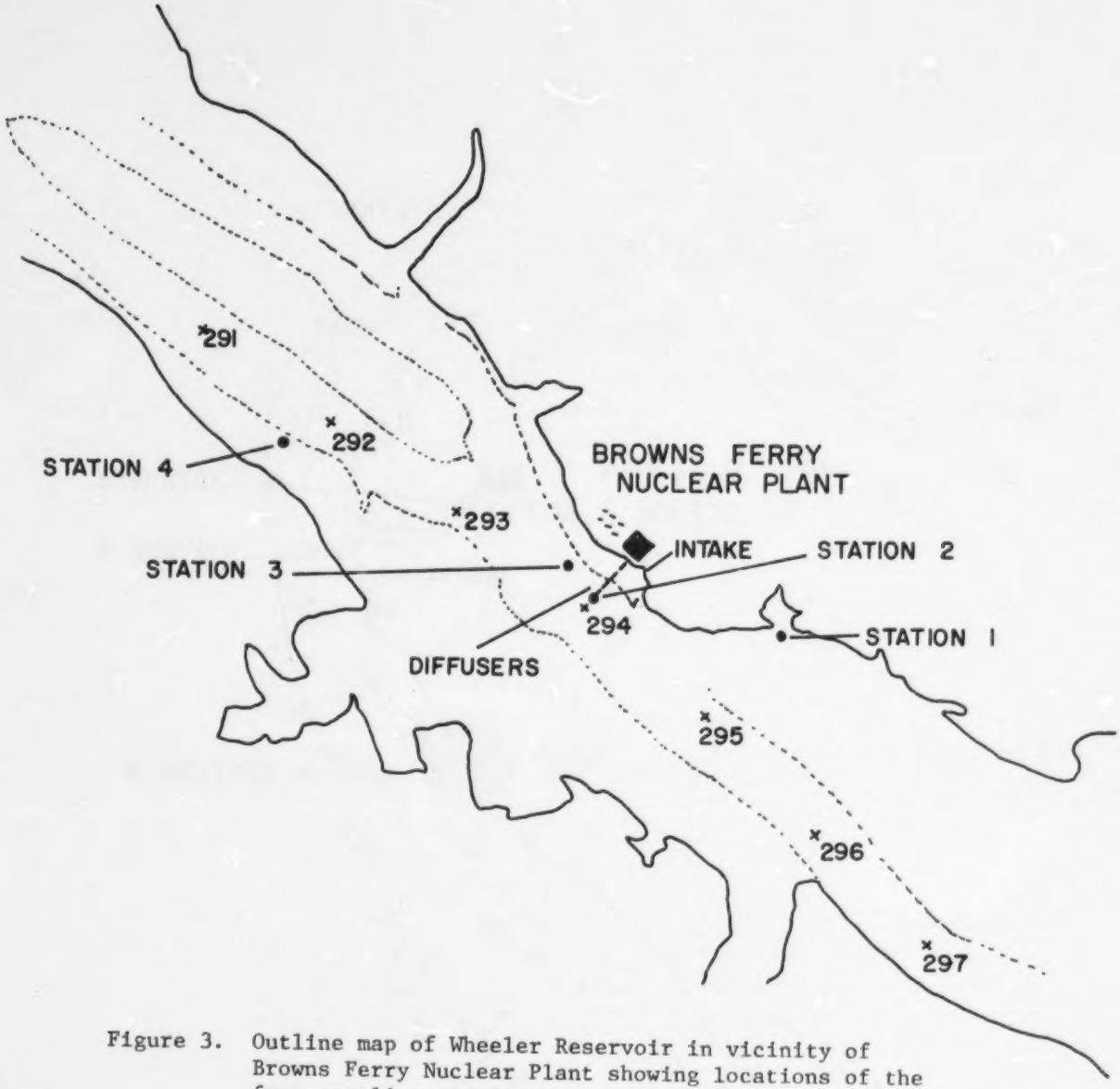


Figure 3. Outline map of Wheeler Reservoir in vicinity of Browns Ferry Nuclear Plant showing locations of the four sampling stations.

SECTION 5

RESULTS AND DISCUSSION

THERMAL TOLERANCE OF IMMATURE STAGES

Chironomus crassicaudatus

Results of the 6-hr thermal shock tests (immediate and delayed mortality) on larvae of C. crassicaudatus are given in Table 1; the regression lines are shown in Figure 4. Mean TL50 values for larvae acclimated to 15° and 20° were nearly identical based on direct, 6-hr percentages of mortality (37.9° and 37.4°); the mean TL50 value for larvae acclimated to 5° was appreciably lower (35.0°), indicating that tolerance increases slightly as acclimation temperature increases. The theoretical upper temperature tolerance of C. crassicaudatus determined by a temperature tolerance triangle (Figure 5) is 684 degrees Celsius squared. This value is greater than any reported by McErlean et al. (1969) for fish, their greatest value being 676 degrees Celsius squared for mosquitofish. However, our value may be an overestimate, as larvae are probably incapable of acclimating to temperatures much above 30°C or as low as 0°C. Therefore, a more realistic estimate would be 550-600 degrees Celsius squared.

Larvae surviving the 6-hr tests were observed for 10 days for posttreatment mortality. In all replicates, posttreatment mortality increased as shock temperature increased (Table 1). Mean posttreatment TL50 values ranged from 29.3° at an acclimation temperature of 5° to 36.1° at an acclimation temperature of 20°. However, mortality was quite high in the controls, indicating that laboratory conditions were not optimal for this species.

Hexagenia bilineata

The percentages of mortality for large nymphs exposed to 6-hr shocks and observed after a 4-week posttreatment period at each acclimation temperature are presented in Table 2. Mortality from the 6-hour shocks increased sharply within a small temperature interval (from 33° to 36°C for the 5°C acclimation group, from 37° to 40°C for the 15°C acclimation group, and from 40° to 43°C for the 25°C acclimation group.) This rapid response agrees with the findings of Sherberger et al. (1975) on two other species of mayflies. The 6-hr responses for the three highest shock temperatures are shown in Figure 6, along with

TABLE 1. DIRECT AND DELAYED MORTALITY OF C. CRASSICAUDATUS LARVAE ACCLIMATED TO 5°, 15°, AND 20°C AND EXPOSED TO 6-HR THERMAL SHOCKS

Acclimation Temp (°C)	Replicate No.	N	Test Temp (°C)	WT	% Mortality (6-hr)	% Final Mortality (10 days)
5°	1	20	5	0	0	25
		20	31	26	0	80
		20	34	29	10	70
		20	37	32	90	90
	2	20	5	0	0	40
		20	31	26	0	70
		20	34	29	10	70
		20	37	32	80	90
	3	20	5	0	0	70
		20	31	26	0	60
		20	34	29	0	80
		20	37	32	95	100
				Mean TL50	35.0°	29.3°
15°	1	10	15	0	0	30
		10	34	19	0	20
		10	37	22	0	70
		10	38*	23	0	80
	2	20	15	0	5	25
		20	34	19	0	45
		20	37	22	25	80
		20	40	25	100	100
	3	16	15	0	0	56
		16	34	19	0	62.5
		16	37	22	0	87.5
		16	40	25	100	100
				Mean TL50	37.9°	35.0°

Table 1. (continued)

Acclimation Temp (°C)	Replicate No.	N	Test Temp (°C)	WT	% Mortality (6-hr)	% Final Mortality (10 days)
20°	1	13	20	0	0	23
		13	37	17	8	46
		13	40	20	100	100
		13	43	23	100	100
	2	20	20	0	10	30
		20	33	13	5	45
		20	36	16	5	35
		20	39	19	100	100
	3	10	20	0	0	30
		10	33	13	0	20
		10	36	16	10	30
		10	39	19	60	80
				Mean TL50	37.4°	36.1°

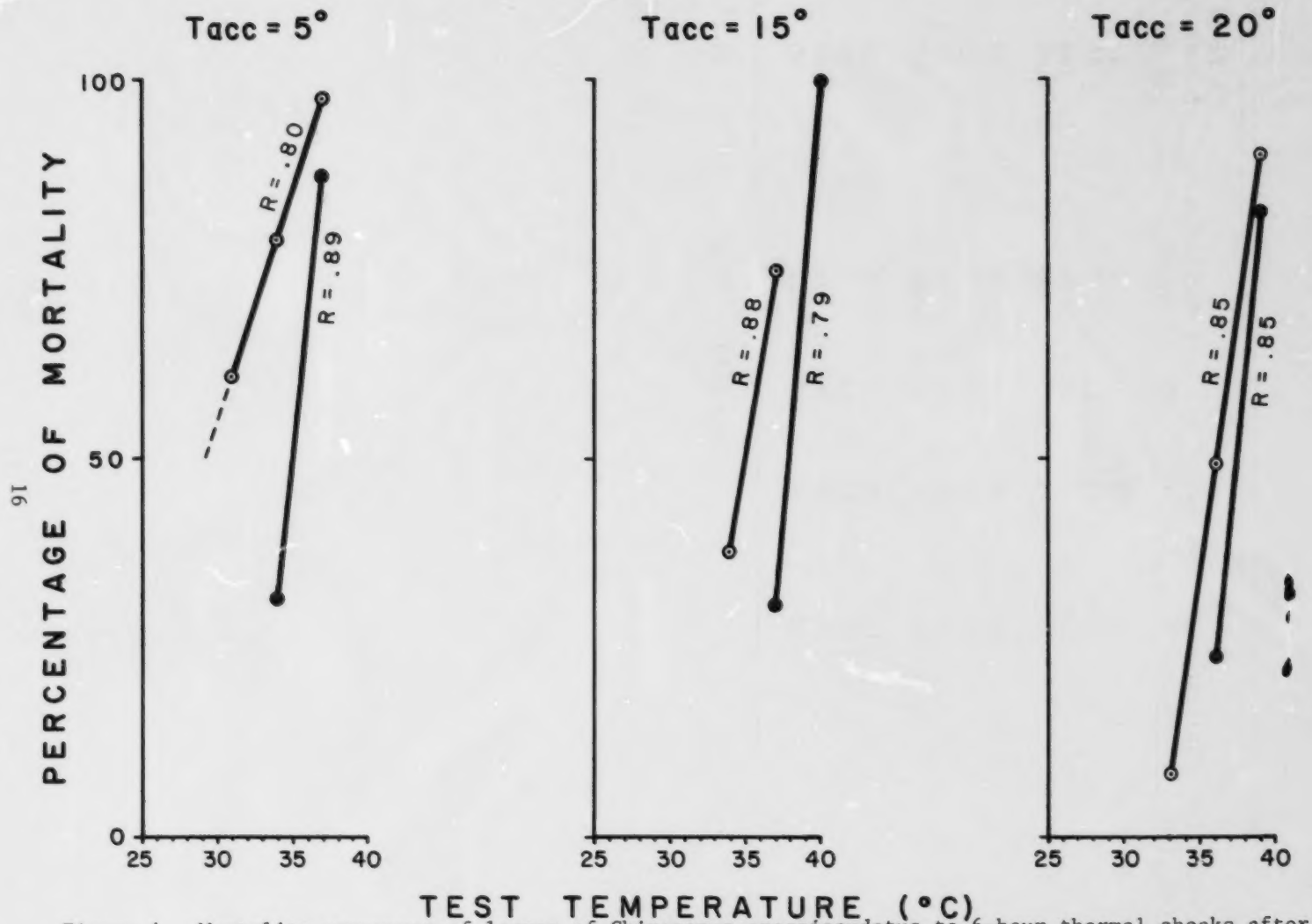


Figure 4. Mortality responses of larvae of Chironomus crassicaudatus to 6-hour thermal shocks after acclimation at three temperatures (5°, 15°, 20°C). Solid circles indicate predicted values of direct (6-hour) mortality, open circles indicate delayed mortality after posttreatment period of 10 days.

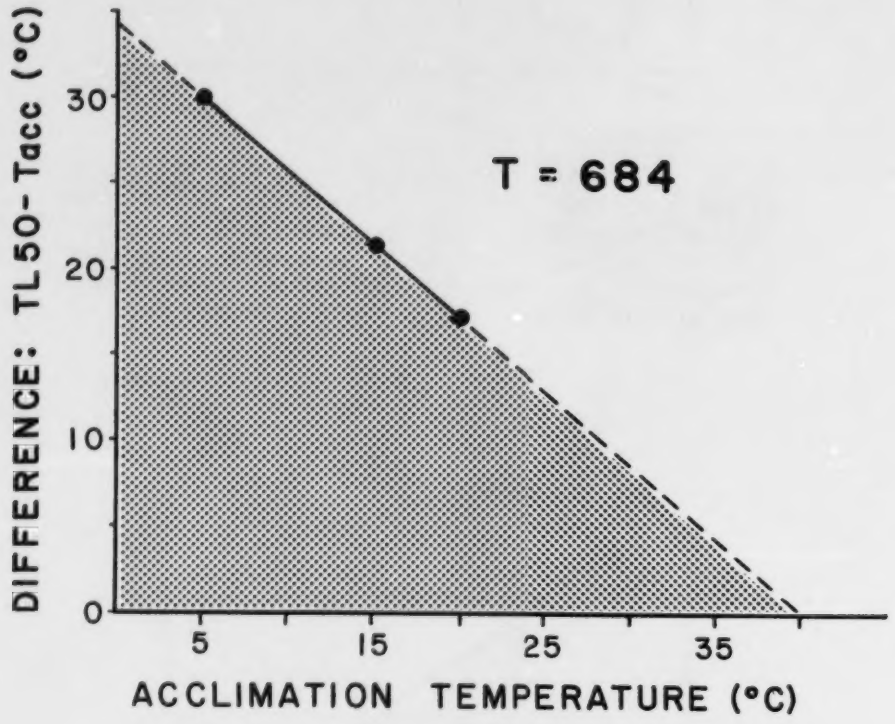


Figure 5. Temperature tolerance triangle for larvae of Chironomus crassicaudatus from Wheeler Reservoir (TRM 298), Limestone County, Alabama; based on 6-hr exposure to elevated temperature. T = area of triangle in degrees Celsius squared.

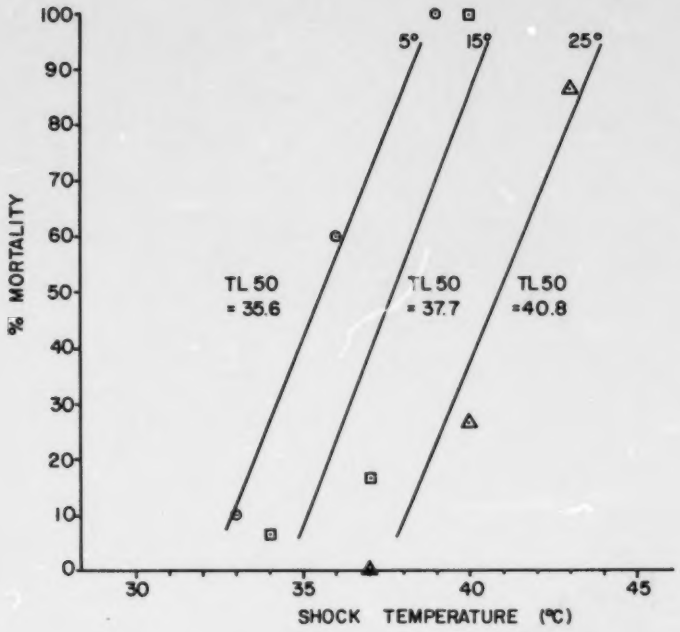


Figure 6. Mortality responses of Hexagenia bilineata nymphs subjected to 6-hour temperature shocks after acclimation to 5°C (circles), 15°C (squares), and 25°C (triangles). Computed regressions lines are indicated by acclimation temperature. TL50 values were calculated from the regression equations.

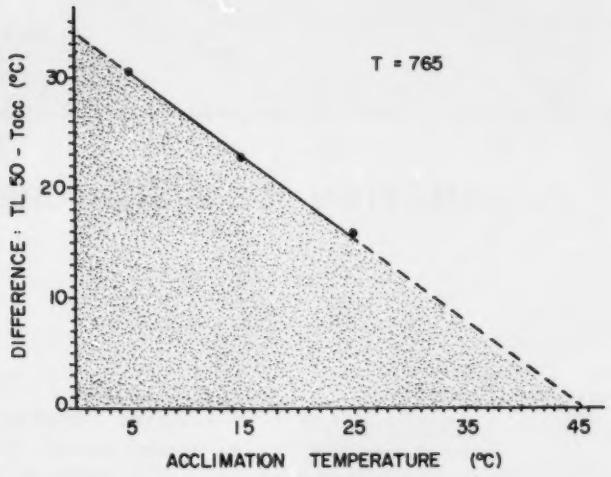


Figure 7. Temperature tolerance triangle for Hexagenia bilineata from Spring Creek embayment, TRM 283.0, Lawrence County, Alabama, based on 6-hour exposures to elevated temperatures. T is area of triangle in degrees Celsius squared.

computed regression lines. The 95 percent confidence limits are not shown, as they exceeded 0 and 100 percent mortality for most of the predicted values because of the low number of observations (3) and degrees of freedom (1). The TL50 values, calculated from the regression equations, increased as acclimation temperature increased, although the difference between shock temperature and acclimation temperature (ΔT) decreased. The latter relationship and the zone of thermal tolerance, wherein at least half of the mayfly nymphs survived are shown in Figure 7. The shaded triangle is quantified by its area, T = 765 degrees Celsius squared.

The survivors of the 6-hr shock tests, held for 4 weeks at their respective acclimated temperature, showed an increase in percentage of mortality with an increase in shock temperature above the 6-hr percentages (Table 2). Posttreatment TL50 values were 1-6° lower than the 6-hr values. These results indicate that there is a latent effect of short-term, sublethal thermal shock on tolerance of H. bilineata nymphs.

TABLE 2. RESULTS OF THERMAL SHOCK TESTS ON NYMPHS OF HEXAGENIA BILINEATA ACCLIMATED TO 5°, 15°, and 25°C. THIRTY NYMPHS WERE EXPOSED TO EACH SHOCK TEMPERATURE

Acclimation Temp. (°C)	Test Temp. (°C)	ΔT	% Mortality (6-hr)	TL50	% Final Mortality (4 wks)	TL50
5°	5	0	0		0	
	30	25	3.3		16.7	
	33	28	10		46.7	
	36	31	60		93.3	
	39	34	100	35.0°	100	33.2°
15°	15	0	0		10	
	31	16	0		3.3	
	34	19	6.7		23.3	
	37	22	16.7		56.7	
	40	25	100	36.9°	100	35.9°
25°	25	0	0		0	
	34	9	0		46.7	
	37	12	0		70	
	40	15	26.7		96.7	
	43	18	86.7	40.4°	100	34.3°

The results of the test on newly-hatched nymphs (first instars) at an acclimation temperature of 25°C indicate that these tiny nymphs are much less tolerant of thermal shock than are larger nymphs (Table 3).

TABLE 3. PERCENTAGES OF MORTALITY OF FIRST-INSTAR NYMPHS OF HEXAGENIA BILINEATA ACCLIMATED TO 25°C AND EXPOSED TO 6-HR THERMAL SHOCKS

Acclimation Temp °C	N	Test Temp (°C)	ΔT	% Mortality Rep. 1	Rep. 2
25°	30	25	0	6.7	10
	30	34	9	36.7	40
	30	37	12	66.7	80
	30	40	15	100	100

TL50 = 35.1

The 6-hr TL50 for first-instar nymphs was 35.1°C compared to 40.4°C for half-grown and larger nymphs (from Table 2, Tacc 25°C).

First instar nymphs were tested only at an acclimation temperature of 25°C because river-bottom temperatures approximate 25° during the season that eggs are laid.

For the laboratory thermal shock tests, an exposure of six hours was chosen because it approximates the relatively short time a drifting nymph may remain in a thermal plume. Nymphs that remain in the river bottom are likely to become slowly acclimated to temperature increases because of the resistance of the mud substrate to rapid temperature changes. Therefore, stationary nymphs probably would not encounter shocks of sufficient magnitude to be acutely lethal under normal electrical generating conditions.

The results of the 6-hour heat-shock tests at different acclimation temperatures show the relatively high level of thermal tolerance of Hexagenia bilineata nymphs. The area of the temperature tolerance triangle (Figure 7) is much larger than that reported by Becker et al. (1975) for a crayfish (T = 424) from two habitats differing in summer maximum temperature (20° and 30°C). As we increased acclimation temperature, the temperature required to kill at least half of the test nymphs increased. Although the short-term thermal maximum of H. bilineata was not determined, it is probably near 40°C. Above this temperature, nymphs would not be able to acclimate and survive even for short periods of time. The rapid response from low to high mortality within a small increment of shock temperature is typical of thermal shock experiments. In general, bottom-dwelling organisms such as Hexagenia bilineata do not experience appreciable diurnal or other short-term temperature fluctuations and are therefore adapted to a relatively uniform thermal regime.

The data gathered from the acclimation tests suggest that seasonal variation in temperature does affect the thermal tolerance of H. bilineata. Nymphs acclimated to winter temperatures may be able to withstand greater ΔT's, although their upper lethal maximum would be lower than in summer. Maximum bottom temperatures below 40°C in summer and 30°C in winter would probably be below acutely lethal levels for larger H. bilineata nymphs (at least half-grown). However, other stages, such as eggs (see Tennessen and Miller 1978, and in press) and small nymphs are more sensitive. And even though elevated temperatures may not be directly lethal, chronic effects may occur. Sublethal temperatures have been reported to alter growth and developmental rates of aquatic insects (Biever 1967, Brittain 1973, Howe 1967, Ingram and Jenner 1976, Mackey 1977, Mokry 1976, Pajunen and Sundback 1973, Rupprecht 1975), as well as their physiology (Csoknya 1973, Philipson and Moorhouse 1976, Tombes and Ingram 1976, Tonapi and Rao 1977), behavior (Nebeker 1971, Philipson and Moorehouse 1974), reproductive processes (Raminani 1975), morphology (Cupp and Horsfall 1970), abundance (Esterly 1975, Hudson 1976, Lenat 1976, Massengill 1976, Tombes and Ingram 1976), and distribution (Hudson 1976, Kromrey et al. 1976). Hutchison (1975) stressed the need for multifactorial experiments to be able to predict ecological impacts of thermal alteration in the environment. Predictive assessment will depend on extrapolation of laboratory results to field situations.

THERMAL TOLERANCE OF EGG STAGE

Effect of Short-Duration Thermal Shock on Hatching of Hexagenia Bilineata Eggs

The percentages of hatching of H. bilineata eggs exposed to the short-duration thermal shocks are given in Table 4. The mean percentages of hatching in response to the three main treatments--shock temperature, shock duration, and hatching day--are shown in Figure 8. Mean cumulative percentage of hatching was highest in the control ($\Delta T = 0^{\circ}$) (Figure 8A). Shock duration did not significantly affect hatching (Figure 8B). Percentage of hatching increased with time (Figure 8C), as expected, to a cumulative mean of 93 percent on day four.

The percentages of hatching were analyzed by a 3-factor analysis of variance, summarized in Table 5. The main effects, except from shock duration, were highly significant ($P = 0.0001$). All interactions were significant, indicating that the various combinations of levels within the three factors affect the hatching response of H. bilineata eggs differently.

Because the increase in percentage of hatching on successive hatching days (Figure 8C) appeared to mask the effects of shock temperature and duration, an analysis of variance was performed using the percentages of hatching from day four only. The results of this analysis and the partitioning of the two main effects using single degree of freedom comparisons are given in Table 6. Shock temperature, averaged over the various shock durations, showed a highly significant linear response,

TABLE 4. PERCENTAGES OF HATCHING OF HEXAGENIA BILINEATA EGGS EXPOSED TO THERMAL SHOCKS (ΔT's OF 5°, 10°, and 15°C) FOR THREE DURATIONS (5, 10, AND 15 MIN) EIGHT HOURS AFTER OVIPOSITION

Pulse Temp. (°C)	Pulse Duration (min)	Date Hatched	Hatching Percentage (%) by Replicate No. 1	2	3	Avg. %
26	5	6/30/79	45.0	48.3	48.0	47.1
(ΔT = 0)		7/01/79	92.7	92.0	93.0	92.6
		7/02/79	95.0	96.0	94.3	95.1
		7/03/79	95.3	94.7	96.7	95.6
	10	6/30/79	49.0	47.7	51.3	49.3
		7/01/79	94.7	92.7	93.3	93.6
		7/02/79	96.7	93.7	96.3	95.6
		7/03/79	97.7	96.0	95.7	96.5
	15	6/30/79	52.0	57.0	54.7	54.6
		7/01/79	90.0	92.0	93.7	91.9
		7/02/79	93.7	94.7	94.3	94.2
		7/03/79	94.0	95.7	97.0	95.6
31	5	6/30/79	23.3	25.7	26.7	24.9
(ΔT = 5		7/01/79	90.7	86.0	90.0	88.9
		7/02/79	92.7	93.7	91.7	92.7
		7/03/79	90.7	92.0	92.0	91.6
	10	6/30/79	21.3	27.3	20.0	23.0
		7/01/79	87.3	84.3	90.0	87.2
		7/02/79	91.0	91.7	94.7	92.5
		7/03/79	93.7	95.0	93.0	93.9
	15	6/30/79	36.0	34.3	28.3	32.9
		7/01/79	87.7	92.7	88.7	89.7
		7/02/79	90.7	93.0	90.3	91.3
		7/03/79	92.7	93.7	91.0	92.5
36	5	6/30/79	15.0	9.7	7.7	10.8
(ΔT = 10)		7/01/79	77.3	79.0	79.7	78.7
		7/02/79	92.0	90.0	85.0	89.0
		7/03/79	92.7	93.3	91.3	92.4
	10	6/30/79	8.7	11.3	10.7	10.2
		7/01/79	80.0	78.7	85.0	81.2
		7/02/79	92.7	87.0	88.0	89.2
		7/03/79	92.0	92.3	93.0	92.4

(continued)

TABLE 4. (continued)

Pulse Temp. (°C)	Pulse Duration (min)	Date Hatched	Hatching Percentage (%) by Replicate No. 1	2	3	Avg. %
36 (Continued)	15	6/30/79	17.0	13.0	12.7	14.2
		7/01/79	76.7	75.0	78.0	76.6
		7/02/79	90.7	89.3	91.3	90.4
		7/03/79	91.0	91.7	93.3	92.0
41 ($\Delta T = 15$)	5	6/30/79	22.0	25.7	25.3	24.3
		7/01/79	80.7	83.7	80.7	81.7
		7/02/79	91.0	88.0	90.7	89.9
		7/03/79	95.0	90.7	91.3	92.3
	10	6/30/79	22.7	21.7	18.7	21.0
		7/01/79	80.3	80.0	80.3	80.2
		7/02/79	89.3	93.3	89.7	90.8
		7/03/79	92.7	93.3	90.7	92.2
	15	6/30/79	16.7	16.0	20.0	17.6
		7/01/79	78.7	80.7	75.3	78.2
		7/02/79	87.0	88.3	85.7	87.0
		7/03/79	90.3	89.0	88.3	89.2

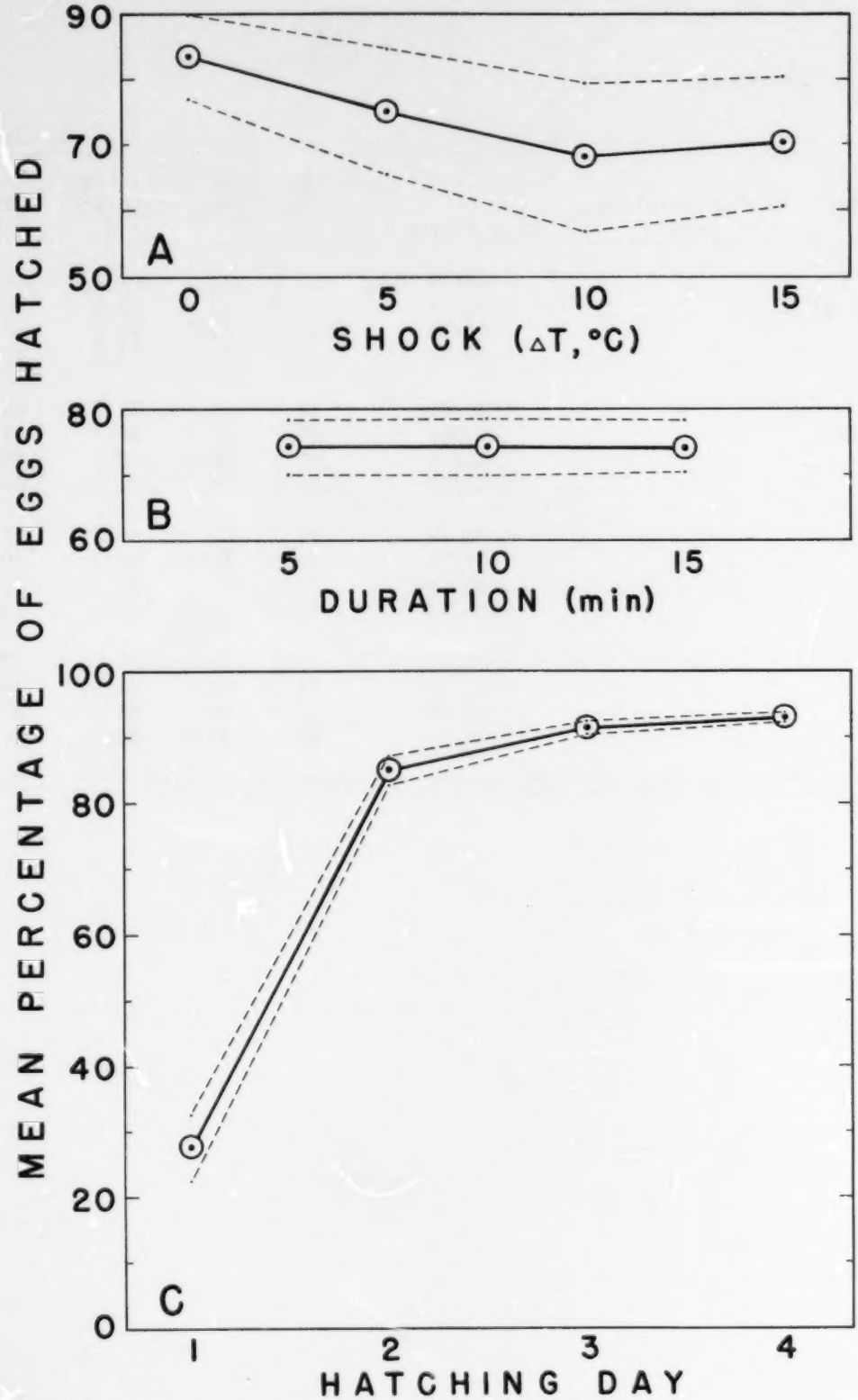


Figure 8. Mean percentages of hatching of Hexagenia bilineata eggs in response to main effects of shock temperature (A), shock duration (B), and hatching day (C). Dotted lines represent 95 percent confidence limits.

TABLE 5. ANALYSIS OF VARIANCE OF THREE-FACTOR THERMAL SHOCK EXPERIMENT ON EGGS OF HEXAGENIA BILINEATA

Source of Variation	df	Mean Square	F	P
Replications	2			
Shock temp., T (t=4)	3	1677.96	412.75**	0.0001
Shock duration, D (d=3)	2	0.04	0.01	0.9903
Hatching day, H (h=4)	3	35331.50	8691.04**	0.0001
TD (t-1)(d-1)	6	27.08	6.66**	0.0001
TH (t-1)(h-1)	9	436.12	107.28**	0.0001
DH (d-1)(h-1)	6	22.83	5.62**	0.0001
TDH (t-1)(d-1)(h-1)	18	9.04	2.22**	0.0068
Error	96	4.065		

TABLE 6. ANALYSIS OF VARIANCE AND PARTITIONING OF TWO MAIN TREATMENTS, SHOCK TEMPERATURE (ΔT) AND SHOCK DURATION (MIN), ON HATCHING SUCCESS OF H. BILINEATA EGGS ON THE FOURTH DAY OF HATCHING

Source of Variation	df	Mean Square	F	P
Shock temp., T (t=4)				
Treatments	3	35.67	17.09**	<0.001
Replications	8	2.14	1.03	>0.10
Error	24	2.087		
Partitioning Treatments:				
Linear response	1	90.60	43.35**	<0.001
Quadratic response	1	10.78	5.16*	0.032
Cubic response	1	5.65	2.70	>0.10
Shock duration, D (d=3)				
Treatments	2	6.32	3.66*	0.05
Replications	11	11.24	6.50**	<0.001
Error	22	1.728		
Partitioning Treatments				
Linear response	1	2.67	1.54	>0.10
Quadratic response	1	9.97	5.77*	0.025

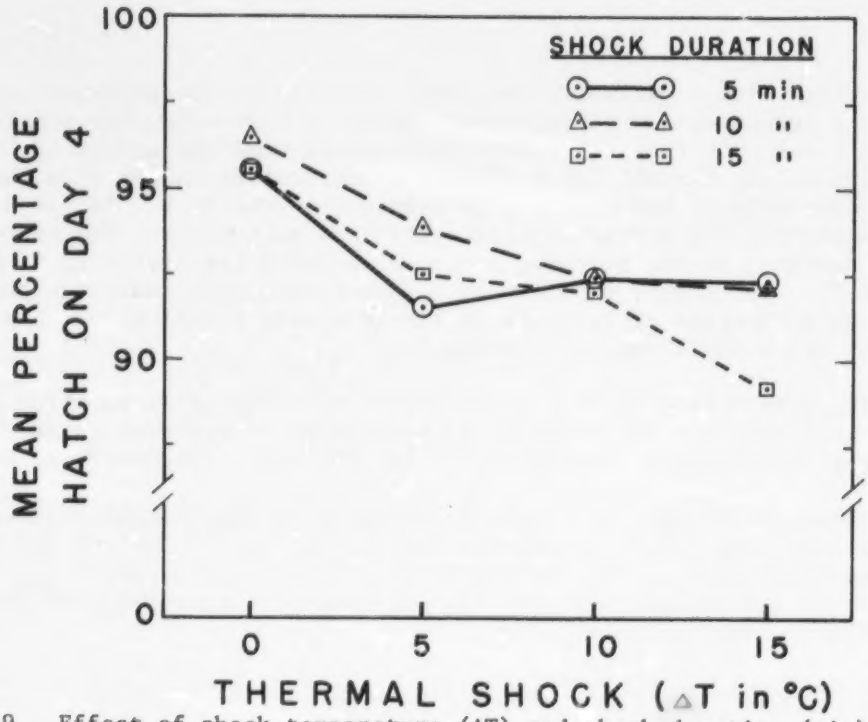


Figure 9. Effect of shock temperature (ΔT) and shock duration (min) on the mean cumulative percentage of hatching of Hexagenia bilineata eggs.

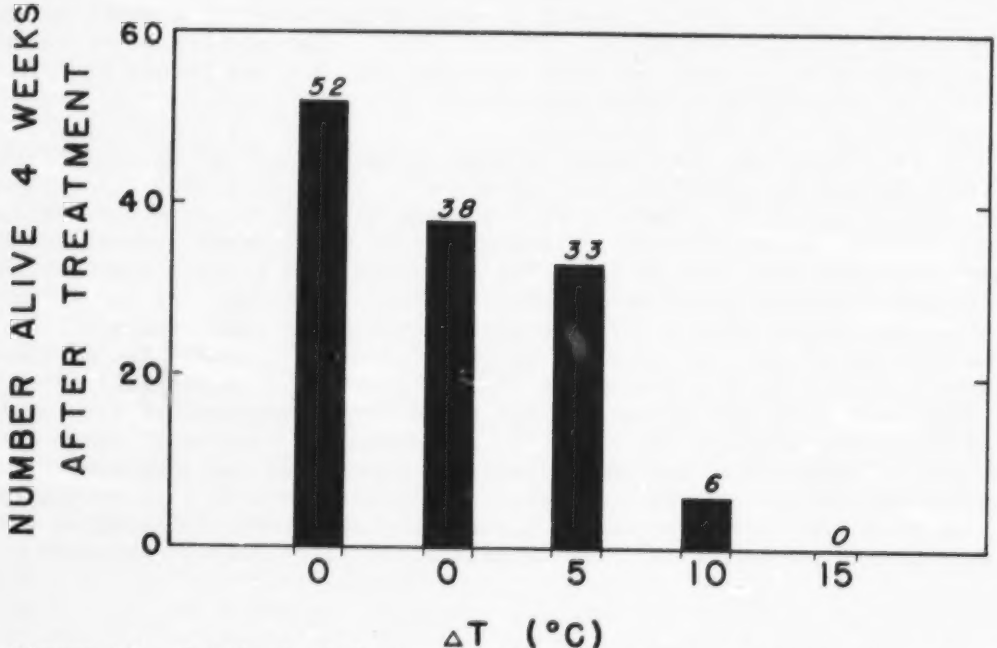


Figure 10. Survival of Hexagenia bilineata nymphs 4 weeks after exposure to short duration (15 min) thermal shocks (ΔT) in the egg stage.

plus a significant quadratic response. Therefore, the decrease in hatching percentage as ΔT increased (shown in Figure 8A) was significant. Sheffe's multiple comparison procedure showed that the percentage of hatching in the control ($\Delta T = 0^{\circ}$) was significantly larger than the other percentages, but that the percentages in the ΔT 5°, 10°, and 15° treatments did not differ significantly from each other. The effect of shock duration on the hatching response on day 4 was also significant (Table 6). The highly significant quadratic response indicates that the mean percentage of hatching in the 10-minute treatment was higher than in the 5-and 15-minute treatments.

The interaction between shock temperature and shock duration is shown in Figure 9. The decrease in percentage of hatching as shock temperature increased, especially in the 15-minute treatment, is evident.

After four weeks, survival of 100 newly-hatched nymphs from each of the four temperature treatments was greater in the control and the 5° treatments than in the two higher ΔT's (Figure 10), indicating that very brief thermal shock in the egg stage can affect subsequent nymphal development even though eggs hatch.

Effect of Constant Temperature on Argia spp. Egg Development

The results of the experiment on eggs and newly-hatched nymphs of Argia moesta are presented in Table 7. Percentage of hatching at 31° and 34° was slightly less than at the ambient temperature (28°), and much lower at 37° and 40°. Increasing incubation temperature resulted in shorter times of development, as evidenced by the greater proportion of eggs which had hatched on September 6 up to 37°C. The few individuals hatching at 40° died in the pronymph stage (first instar), which is usually succeeded by a moult to the second instar within a few minutes (Corbet 1963). The percentage of survival of the nymphs was low after one week, as expected, but was lowest (5 percent or less) at the two higher temperatures.

The results of the experiment on Argia translata are given in Table 8. Unlike A. moesta, no hatching occurred at 40°C; also, no eggs hatched in one of the replicates at 37°, whereas 61.3 percent hatched in the other replicate. The percentages varied between replicates at the other temperatures, but were higher on the average than at 37°. The two replicates at each temperature also showed different patterns of hatching from day to day. As for A. moesta, an incubation temperature of 37° decreased development time, and appeared to shorten the period during which the majority of eggs hatched. The percentage of survival of the nymphs hatched at 28° was greater (25.5 percent) after one week than that of nymphs hatched at the three higher temperature treatments (6.5-7 percent). Despite the variation in responses, results of these two experiments suggest that the upper limit for successful egg development in these two species is near 34°. However, the lower survival rate of nymphs hatching from elevated temperatures indicates that latent, detrimental effects at temperatures exceeding 34°C can be manifested after initial exposure.

TABLE 7. HATCHING SUCCESS AND SURVIVAL OF HATCHLINGS OF ARGIA MOESTA EXPOSED TO FIVE CONSTANT TEMPERATURES DURING EGG STAGE (AUGUST 28 TO SEPTEMBER 9, 1979)

	Number of Nymphs Hatched at Incubation Temperature (°C)				
Date	28° (ambient)	31°	34°	37°	40°
9/6	0	45	67	99	6
9/7	13	28	52	2	2
9/8	64	30	4	0	2
9/9	47	0	0	0	0
Total No. Hatchlings	124	103	123	101	10
Total No. of Eggs Exposed	139	159	152	295	140
Percent Hatched	89.2	64.8	80.9	34.2	7.1
No. of Hatchlings Alive After 1 week	20	8	21	5	0
Percent Survival	16	8	17	5	0

TABLE 8. HATCHING SUCCESS AND SURVIVAL OF HATCHLINGS OF ARGIA TRANSLATA EXPOSED TO FIVE CONSTANT TEMPERATURES DURING EGG STAGE (SEPTEMBER 12 TO SEPTEMBER 28, 1979)

	Number of Nymphs Hatched at Incubation Temperature (°C)									
Rep.	28° (ambient)		31°		34°		37°		40°	
Date	1	2	1	2	1	2	1	2	1	2
9/20	0	0	2	0	260	82	3	0	0	0
9/21	0	0	26	436	195	309	156	0	0	0
9/22	0	0	42	347	24	46	59	0	0	0
9/23	235	2	30	103	8	21	25	0	0	0
9/24	41	129	23	92			25	0	0	0
9/25	40	212						0	0	0
9/26	x	x						0	0	0
9/27	121	213						0	0	0
9/28	9	52						0	0	0
Total No. Hatchlings	446	608	123	978	487	458	268	0	0	0
Total No. of Eggs Exposed	594	668	339	1185	557	484	437	1433	980	627
Percent Hatched	75.1	91.0	36.3	82.5	87.4	94.6	61.3	0	0	0
No. of Hatchlings Alive After 1 Week	269 / 1054		77 / 1101		61 / 945		18 / 268		___	
Percent Survival	25.5		7.0		6.5		6.7		-	

x = no count taken.

POPULATION STRUCTURE OF *HEXAGENIA BILINEATA* IN THERMAL PLUME AREAS

Vertical Temperature Profiles

At Johnsonville Steam Plant, the 1978 profile trends (Figure 11) were similar to those of the previous year (Tennessen and Miller 1978), in that the thermal plume at stations 3 and 4 extended to the river bottom only during the cold part of the year (November to March). River bottom temperatures were near or only slightly above ambient from April 21 to October 26; during this period, ambient river temperature was above 15°C. The greatest surface ΔT recorded between station 1 (upstream from plant) and station 3 (in discharge canal) was 6.6°C on October 26, whereas the greatest bottom ΔT was 6.3°C on November 29, 1978.

The profiles at Gallatin Steam Plant (Figure 12) differed from those at Johnsonville in that the thermal plume extended to the river bottom at stations 3 and 4 on each recording date. These stations are shallower than the thermal discharge station at Johnsonville. At station 5, the thermal plume was confined to the upper one to two meters of the water column. The greatest surface ΔT recorded at station 4 was 7.0°C, on March 24; the greatest bottom ΔT at this station was 6.7°C, on March 24 and again on June 28.

At Browns Ferry Nuclear Plant, the temperature profiles at the four stations were very similar (Figure 13). Comparatively low ΔT's, ranging from zero to 3.7°C, were recorded downstream of the thermal discharge (station 3). On April 25 and June 29 the water temperatures at station 3 were slightly lower than those at station 1.

Comparisons of bottom temperature readings at the stations from which mayfly nymphs were collected are graphically shown in Figure 14. The thermal station at Gallatin was consistently warmer than the control (ΔT = 2.2°-6.7°C) compared to the more nearly equal temperatures recorded at the other plants throughout most of the year. These bottom temperatures indicate that mayfly nymphs within the influence of the thermal plume from Gallatin Steam Plant were exposed to a consistently higher thermal regime than those at the other two plants (Figure 15). Mayflies downstream from Browns Ferry Nuclear Plant apparently experienced lower ΔT's but a more variable thermal regime than those below Johnsonville Steam Plant. Factors which contribute to variations in thermal plume conditions include season, station depths, plant output, flow conditions, and at Browns Ferry Nuclear Plant, the extent of usage of the cooling towers.

Sediment Analyses

Percentage of composition for various particle sizes and percent organic matter (total volatile solids) are given in Table 9. These data indicate that the sediments at the selected stations were fairly similar. Notable differences occurred between the Johnsonville stations

in phi sizes <4 to 4 (sand) and 7 (fine silt), and 8 (very fine silt) and between the Browns Ferry stations in phi size 6 (medium silt). Percent organic matter was relatively high at all six stations.

TABLE 9. COMPOSITION OF PARTICLE SIZES (%), WITHIN SEDIMENT SAMPLES TAKEN IN APRIL 1978, FROM SELECTED STATIONS AT JOHNSONVILLE STEAM PLANT, GALLATIN STEAM PLANT, AND BROWNS FERRY NUCLEAR PLANT (BASED ON PHI SCALE DEVISED BY KRUMBEIN)

	Ø (Phi) Size Classes								
	F4-4	5	6	7	8	9	10	J10	
Station	Sand	Coarse Silt	Med. Silt	Fine Silt	Very Fine Silt	Clay	Clay	Clay	% Organic Matter
Johsonville									
Station 1	34.1	12.1	19.7	10.2	4.0	3.3	3.1	13.5	22.06
Station 3	5.5	9.8	26.2	20.0	9.8	5.2	5.0	18.5	28.57
Gallatin									
Station 1	6.1	19.4	26.5	22.9	0.2	4.5	4.5	15.9	25.95
Station 4	10.3	18.2	28.9	16.6	5.9	3.9	3.6	12.6	27.15
Browns Ferry									
Station 1	1.8	5.2	28.8	19.5	10.1	6.5	6.3	21.8	28.48
Station 3	6.4	10.6	17.1	16.2	8.9	7.4	6.4	27.0	29.39

Population Dynamics of Hexagenia bilineata

Population Estimates--

In general, the mean number of nymphs per sample tended to decrease through the spring and early summer, followed by an increase in August (Figures 16-18). An exception to this trend occurred in May at station 1 above Browns Ferry Nuclear Plant, reasons for which are unknown. Emergence, predation, other natural mortality factors, and drift probably account for the population declines, whereas recruitment of young nymphs from eggs laid in June and July increased the populations later in the summer.

Samples from station 3 (discharge canal) near Johnsonville Steam Plant contained extremely low numbers of mayfly nymphs from March through July (Figure 16). The few nymphs found were small compared to those from samples at station 1, whereas samples taken in 1977 revealed that the population at station 3 was comparable in numbers and mean size to that at station 1 (Tennessen and Miller 1978). The cause of the drastic decline in the mayfly population in 1978 is unknown, although

a major oil spill at Johnsonville Steam Plant occurred on February 16 and 17, 1978 (letter from H. G. Moore to J. C. White, April 18, 1978). An estimated 10,000 gallons of No. 2 fuel oil spilled, some of it reaching the river just downstream of the mouth of the discharge canal. A strong northerly wind blew the floating oil upstream into the discharge canal and the plant intake channel (see Figure 1). However, whether the oil reached the discharge canal bottom and if so, to what extent is unknown. Population estimates for August and September indicate rapid recovery at station 3, as numbers were comparable to those at station 1 (Figure 16).

Size Comparisons Based on Head Width--

Mean head widths, along with the number of nymphs measured, the sample variances, the 95 percent confidence intervals and the ranges for each sampling station per sampling date are given in Table 10. A total of 5,026 nymphs were measured for head width.

Mean head width of H. bilineata nymphs in samples from station 3 at Johnsonville was markedly less from March (0.62 mm less) through June (0.33 mm less) than mean head width of nymphs from station 1 (Figure 19). These station differences are further depicted by size class comparisons (Figure 22). Nymphs were present in nearly all size classes at station 1 from March to July in contrast to the patchy distribution at station 3. By August the population at station 3 recovered and growth apparently accelerated as more large nymphs were present than at station 1. A simple t-test of the means on August 29 showed that they were significantly different ($P = < .01$). Differences in size class frequencies recurred in September and October (Figure 22).

Mean head width at station 4 near Gallatin Steam Plant was 0.27 mm greater than at station 1 on May 25 and 0.36 mm greater on June 28 (Table 10 and Figure 20). The number of nymphs present in the July 27 samples was too low to allow a meaningful comparison. Mean head widths at the two stations on August 29 and September 26 samples were very similar. The frequency histograms (Figure 23) show that the populations were fairly similar in structure throughout the sampling period. On May 25 and June 28, a larger percentage of the nymphs at station 4 were in the larger size classes. The converse of this relationship ocurred on August 29.

At stations 1 and 3 near Browns Ferry Nuclear Plant, differences in mean head width were slight (Figure 21). Of the seven sampling dates, the mean at station 3 was greater than at station 1 only on May 26. The distribution of nymphs in the size classes was very uniform between the two stations (Figure 24).

To further compare the development of nymphs at ambient and thermal stations, the number of nymphs possessing darkened wings on each sampling date was recorded and converted to a percentage of the total number collected at that station (Table 11). Because of the perturbation to the population at station 3 near Johnsonville Steam Plant, the results

TABLE 10. RESULTS OF HEAD WIDTH MEASUREMENTS OF HEXAGENIA BILINEATA NYMPHS COLLECTED AT THERMAL AND AMBIENT STATIONS NEAR JOHNSONVILLE AND GALLATIN STEAM PLANTS AND BROWNS FERRY NUCLEAR PLANT, 1978

		N	$\bar{x}$ (mm)	S^2	95% Confidence Interval (mm)	Range (mm)
Johnsonville						
March 23	Sta. 1	194	1.87	0.204	± .064	.99 - 3.16
	Sta. 3	26	1.25	0.173	.168	.89 - 3.16
April 21	Sta. 1	125	1.76	0.166	.072	.89 - 2.86
	Sta. 3	33	1.48	0.090	.106	.99 - 2.27
May 24	Sta. 1	89	1.91	0.203	.095	.99 - 3.06
	Sta. 3	3	1.44	0.090	.551	1.18 - 1.77
June 27	Sta. 1	42	2.42	0.146	.119	1.38 - 2.86
	Sta. 3	16	2.09	0.143	.201	1.28 - 2.66
July 26	Sta. 1	71	1.60	0.240	.116	.99 - 2.66
	Sta. 3	14	1.53	0.089	.171	.99 - 1.97
August 29	Sta. 1	136	1.20	0.140	.064	.59 - 2.27
	Sta. 3	148	1.35	0.323	.093	.64 - 2.81
September 26	Sta. 1	254	1.30	0.138	.046	.44 - 2.37
	Sta. 3	284	1.52	0.234	.056	.59 - 2.86
October 26	Sta. 1	235	1.15	0.163	.052	.39 - 2.42
	Sta. 3	186	1.50	0.239	.070	.64 - 2.86
		1,856				
Gallatin						
March 24	Sta. 1	150	2.00	0.298	.088	.89 - 3.35
	Sta. 4	59	1.88	0.207	.118	.99 - 3.25
April 20	Sta. 1	111	1.87	0.329	.108	.69 - 3.16
	Sta. 4	76	1.96	0.304	.126	.79 - 3.16
May 25	Sta. 1	144	1.81	0.484	.115	.69 - 3.16
	Sta. 4	81	2.08	0.433	.146	.89 - 3.45
June 28	Sta. 1	85	2.06	0.351	.129	.99 - 3.45
	Sta. 4	67	2.42	0.487	.170	.99 - 3.35

(continued)

Table 10. (continued)

		N	$\bar{x}$ (mm)	S^2	95% Confidence Interval (mm)	Range (mm)
July 27	Sta. 1	7	2.87	0.306	.495	1.97 - 3.65
	Sta. 4	31	2.33	0.538	.269	.89 - 3.75
August 29	Sta. 1	38	1.52	0.395	.206	.79 - 2.96
	Sta. 4	68	1.50	0.164	.098	.89 - 2.37
September 26	Sta. 1	31	1.84	0.104	.119	1.18 - 2.61
	Sta. 4	30	2.02	0.189	.162	1.33 - 2.86
		978				
Browns Ferry						
March 27	Sta. 1	169	1.88	0.288	.081	.89 - 3.25
	Sta. 3	214	1.79	0.361	.080	.79 - 3.06
April 27	Sta. 1	166	2.02	0.338	.088	.89 - 3.35
	Sta. 3	180	1.96	0.312	.082	.79 - 3.25
May 26	Sta. 1	281	1.85	0.539	.086	.69 - 3.35
	Sta. 3	181	2.05	0.486	.102	.79 - 3.06
June 29	Sta. 1	100	2.40	0.337	.115	1.12 - 3.55
	Sta. 3	140	2.21	0.537	.122	.89 - 3.25
July 27	Sta. 1	125	1.85	0.360	.106	.89 - 2.96
	Sta. 3	117	1.84	0.280	.097	.89 - 2.86
August 30	Sta. 1	190	1.98	0.211	.065	.94 - 3.16
	Sta. 3	130	1.79	0.331	.100	.54 - 2.76
September 27	Sta. 1	96	2.33	0.136	.075	1.28 - 3.11
	Sta. 3	103	2.05	0.229	.093	.79 - 2.76
		2,192				

N = number of individuals measured in sample
$\bar{x}$ = the sample mean
S^2 = the sample variance

TABLE 11. NUMBER OF H. BILINEATA NYMPHS POSESSING DARKENED WING PADS AT THE AMBIENT AND THERMAL STATIONS NEAR JOHNSONVILLE AND GALLATIN STEAM PLANTS AND BROWNS FERRY NUCLEAR PLANT

	Johnsonville				Gallatin				Browns Ferry			
	Sta. 1		Sta. 3			Sta. 1	Sta. 4			Sta. 1	Sta. 3	
3/23/78	0		0		3/24/78	0	0		3/27/78	0	0	
4/21/78	0		0		4/20/78	0	0		4/27/78	0	0	
5/24/78	0		0		5/25/78	0	0		5/26/78	0	0	
6/27/78	12	(28.6%)	1	(6.3%)	6/28/78	1	(1.2%)	20 (29.9%)	6/29/78	6	(6%)	35 (25%)
7/26/78	2	(2.8%)	0		7/27/78	2	(28.6%)	12 (38.7%)	7/27/78	12	(9.6%)	10 (8.5%)
8/29/78	1	(0.7%)	11	(7.4%)	8/29/78	0	0		8/30/78	0	0	
9/26/78	0		0		9/26/78	0	0		9/27/78	0	0	
10/26/78	0		0									

are not comparable in June and July. With recovery of the population in August, a larger percentage of the nymphs were fully mature at station 3 than at station 1. At Gallatin Steam Plant and Browns Ferry Nuclear Plant in late June, larger percentages of nymphs were mature at the thermal stations. However, the first emergence was observed to occur at Browns Ferry stations 1 and 3 simultaneously during the third week of June, which indicates that development was completed simultaneously. In addition, the size class frequency data indicate that the populations at the Browns Ferry stations were similar in structure (Figure 24, June 29). The first emergence at Gallatin was not observed, although mean head width (Figure 20) and size class data (Figure 23) indicate that on June 28, a larger percentage of the nymphs were near maturity at station 4 than at station 1.

The effects of elevated temperature on organisms in their natural habitat are difficult to assess because of daily and seasonal variation and the presence of numerous other variables which may affect biological processes. Relatively higher temperatures generally increase developmental rates; however, rapid development at high temperatures can result in adult insects that are relatively lighter in weight (Chapman 1971; Vannote and Sweeney 1980). Our comparisons of size of Hexagenia bilineata nymphs inhabiting ambient and thermally-enriched areas near three TVA electric-generating plants indicate that mean size is generally larger at thermal plume stations, especially in May prior to the first adult emergence. These station differences were obvious in samples from the vicinities of Gallatin Steam Plant and Browns Ferry Nuclear Plant (Figures 20 and 21). The histograms (Figures 23 and 24) also show a larger percentage of nymphs present in the smaller size classes at the ambient stations. Because of a major population reduction in the thermal discharge canal near Johnsonville Steam Plant early in 1978, a size comparison was not meaningful. However, in May 1977, mean size was larger at the thermal station (Tennessen and Miller 1978).

Although mean size of H. bilineata nymphs was generally larger at our thermal stations, results of the comparisons were quite variable throughout the sampling period, and significant differences did not always occur. It is plausible that the above-ambient thermal regimes produced by these plants accelerated mayfly growth. However, other variables such as food availability, crowding, and genetic differences between demes affect growth and size. Wright and Mattice (1981) found that size was also dependent on sex, females being larger, and that nymphs reared at their higher temperature (30°C) were smaller in the final instar than nymphs reared at the lower temperatures (22.5 and 15°C). The similarities we found in organic matter and population densities at the ambient and thermal stations suggest that food and crowding did not account for the size differences observed, although the field studies were not designed to isolate the effects of these variables. Size comparisons based on field samples appear to be inadequate for determining the effects of elevated temperature on growth or developmental rates because differently aged individuals are present. Controlled experiments on single-age groups would make

possible comparisons of growth rate, size at maturity and devolopment time under different thermal regimes.

The extent of development of the wing pads of mayfly nymphs is an indicator of maturity. Nymphs with dark, swollen wing pads are near time for emergence to the adult stage. At the Gallatin and Browns Ferry plants, the larger percentages of nymphs with dark wing pads at the thermal stations (June 28 and 29) are evidence of advanced nymphal development, and support the implications drawn from the size comparisons. However, the samples were collected after the first adult emergence, which occurred the third week of June upstream and downstream of Browns Ferry Nuclear Plant. This simultaneous emergence at the two stations is similar to the first emergence in 1977 at the thermal and ambient stations near Johnsonville Steam Plant (Tennessen and Miller 1978). Therefore, although nymphs apparently reached maturity sooner in thermally enriched areas, data on adult emergence indicates that emergence time was not affected. Coutant (1967), Nebeker (1971), and Hudson (1976) reported premature emergence in several species of aquatic insects in response to elevated winter temperatures, and concluded that temperature regulates emergence time. The field and laboratory data presented by Lutz (1968) on the damselfly Lestes eurinus (Say) confirms this conclusion. In contrast, Miyakaua (1969) showed that the dragonfly Pseudothemis zonata (Burmeister) develops at similar rates in the laboratory and field, despite higher winter temperatures in the laboratory. Elvang and Madsen (1973), working on a stonefly, suggested that day length rather than temperature is the regulating factor for successful emergence. Responses appear to be species-specific, and more research is needed to clarify the relationship between extrinsic factors and the development and time of emergence in aquatic insects.

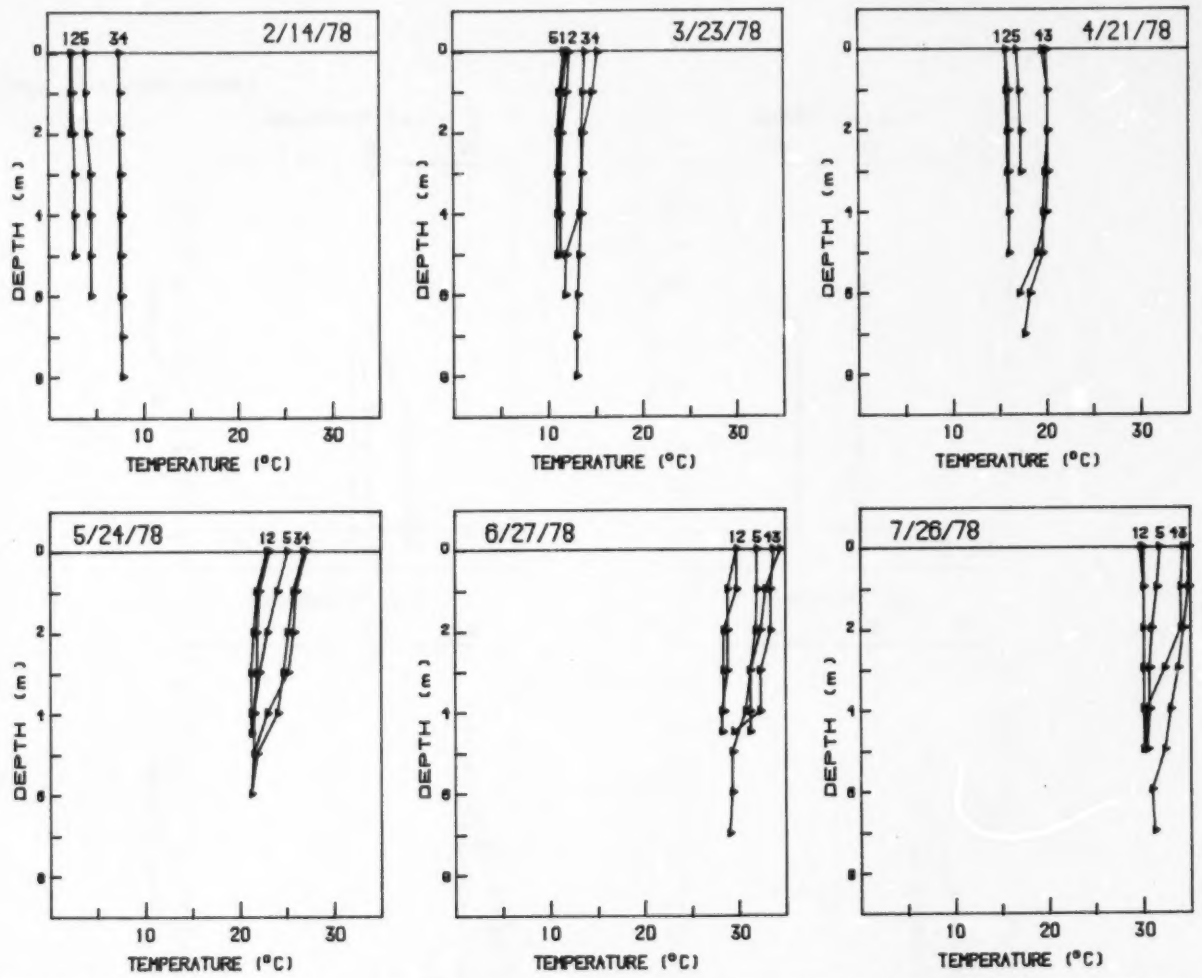


FIGURE 11. Vertical temperature profiles at five stations in vicinity of Johnsonville Steam Plant, Kentucky Lake, from February to November 1978. Numbers at top of profiles refer to stations.

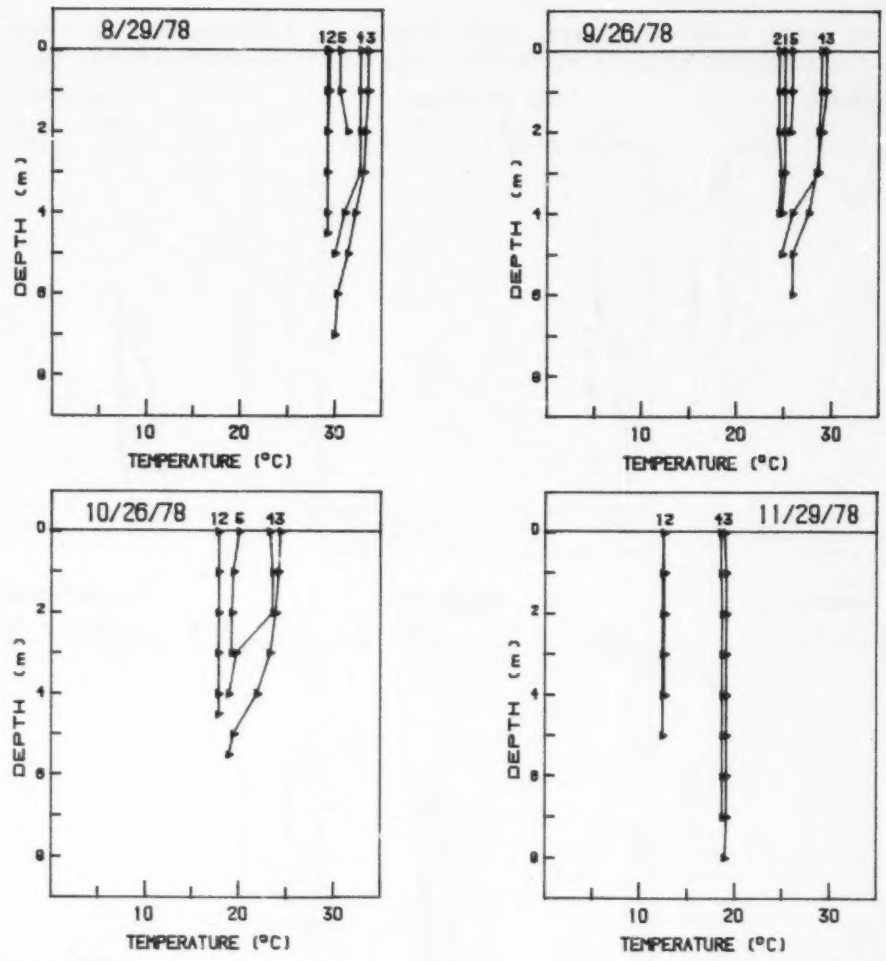


FIGURE 11. (continued).

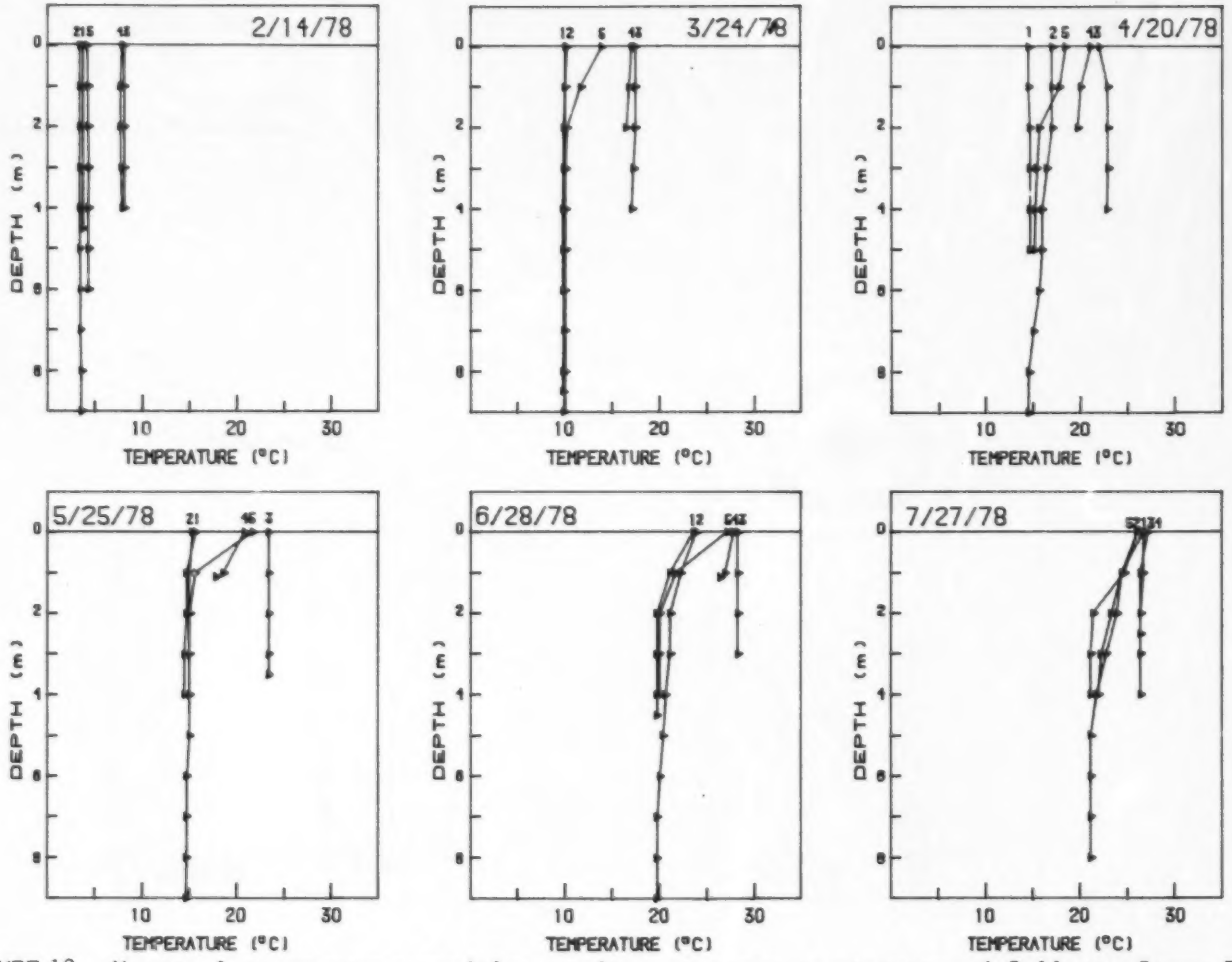


FIGURE 12. Vertical temperature profiles at five stations in vicinity of Gallatin Steam Plant, Cumberland River, from February to September 1978.

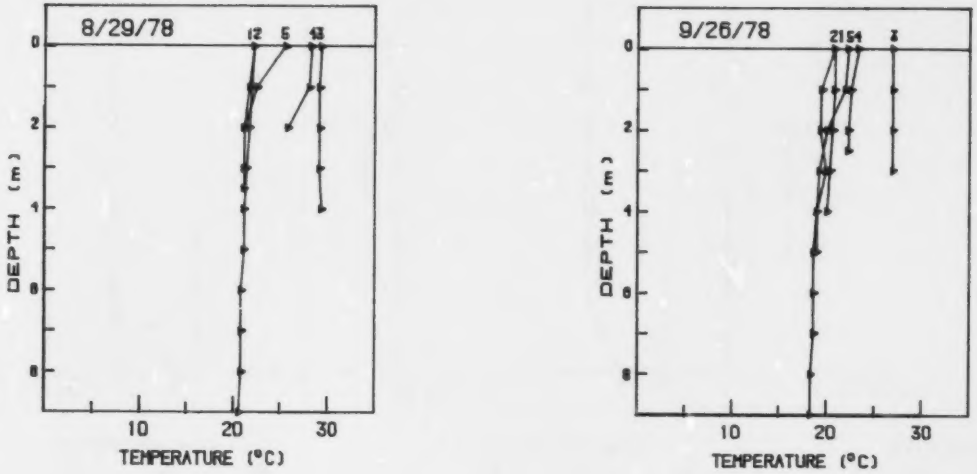


FIGURE 12.(continued).

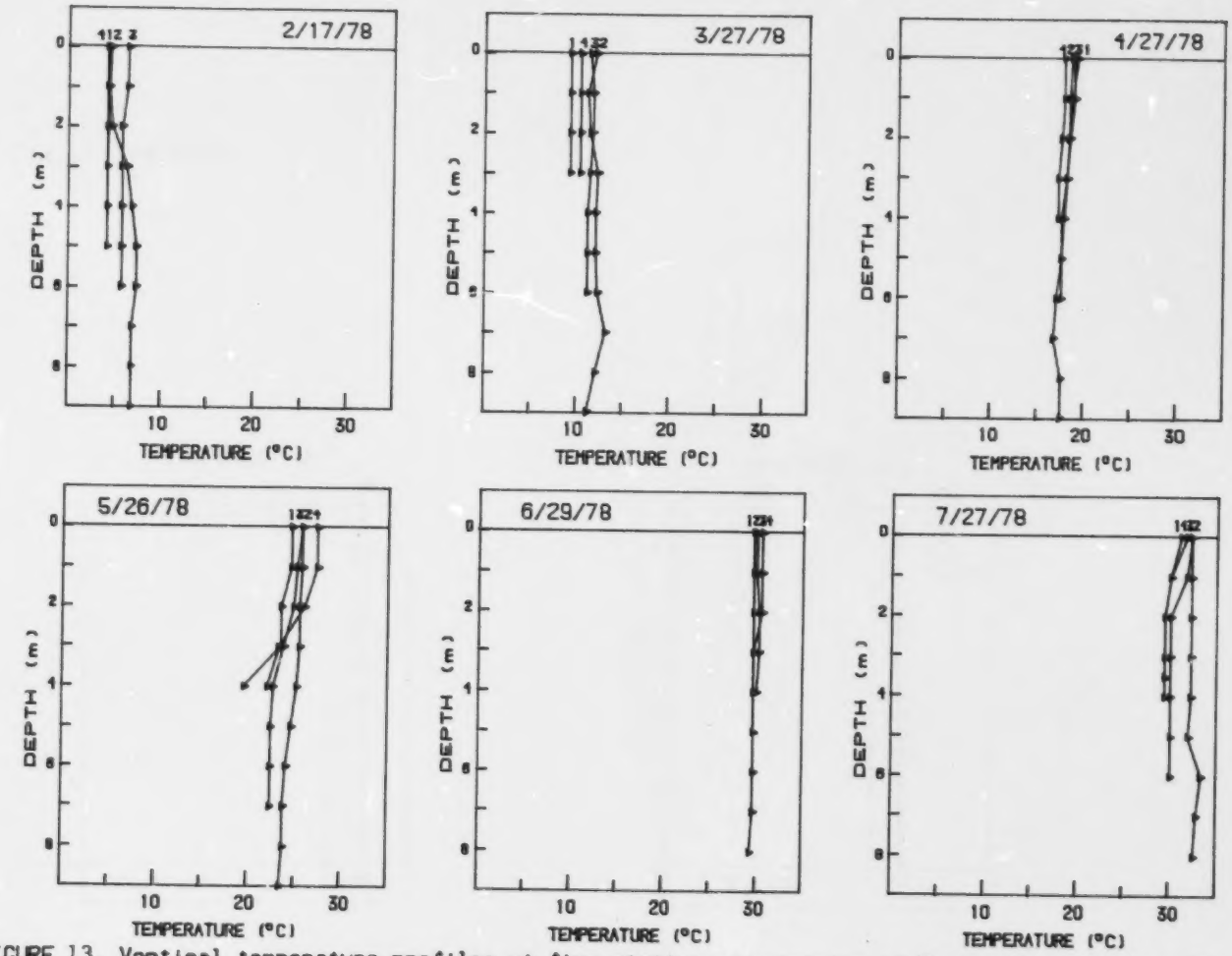


FIGURE 13. Vertical temperature profiles at five stations in vicinity of Browns Ferry Nuclear Plant, Tennessee River, from February to September 1978.

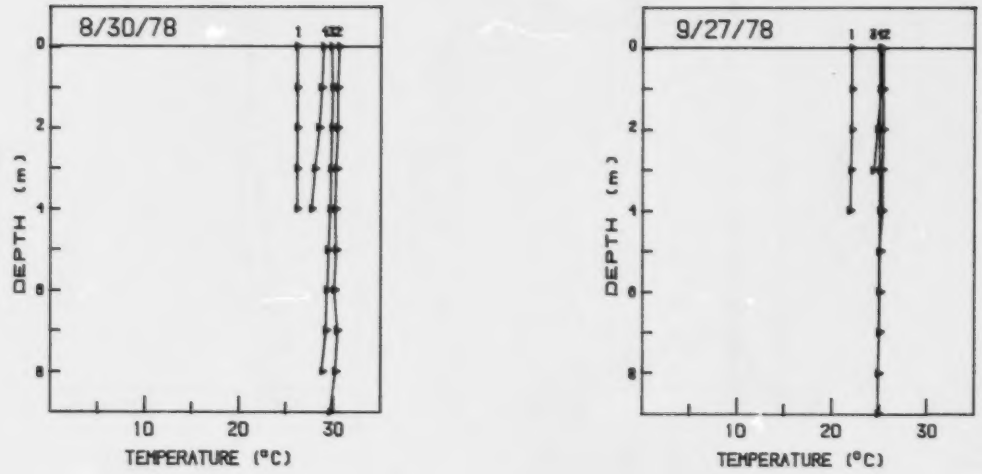


FIGURE 13.(continued).

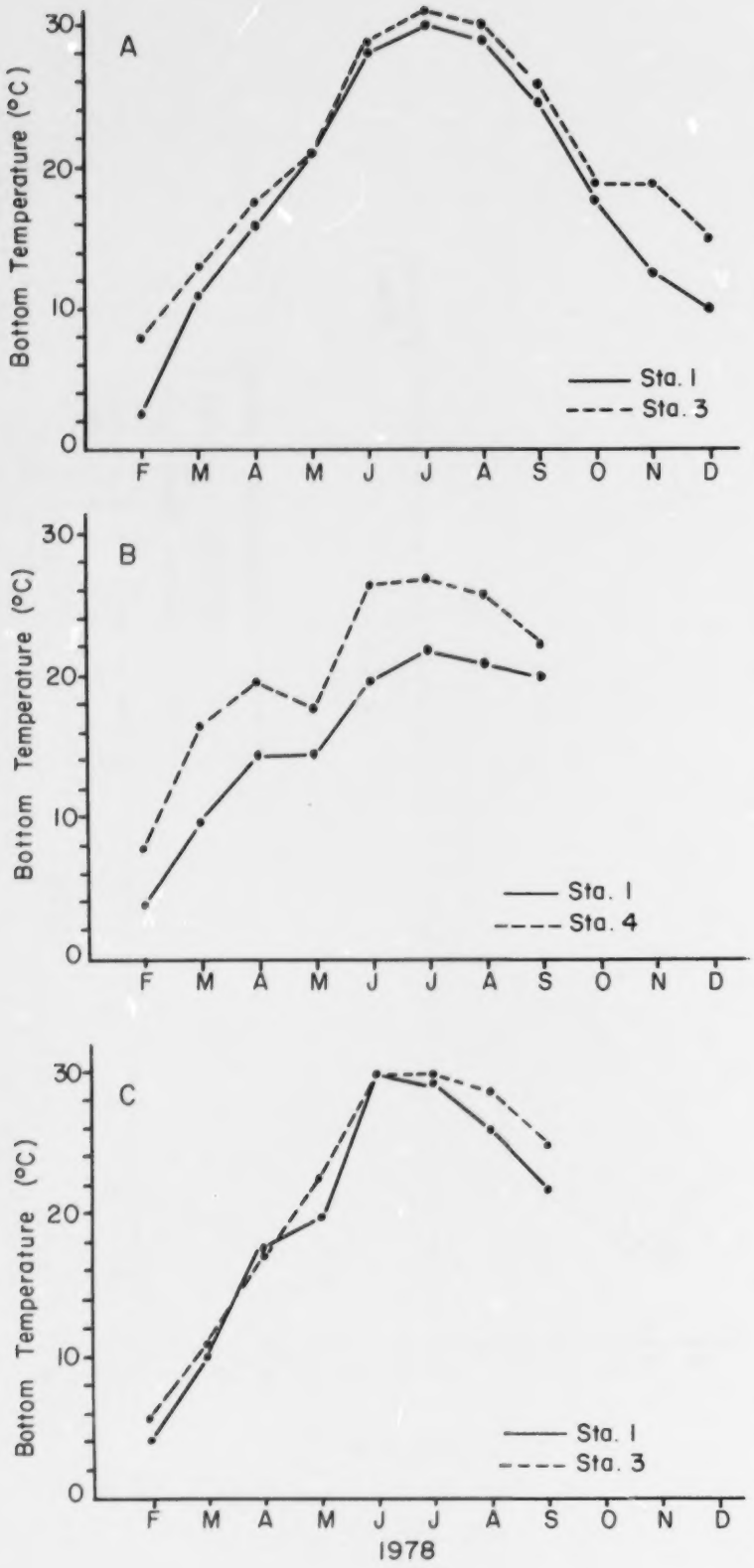


Figure 14. Bottom temperatures at two stations near Johnsonville Steam Plant (A), Gallatin Steam Plant (B), and Browns Ferry Nuclear Plant (C) from February to December 1978.

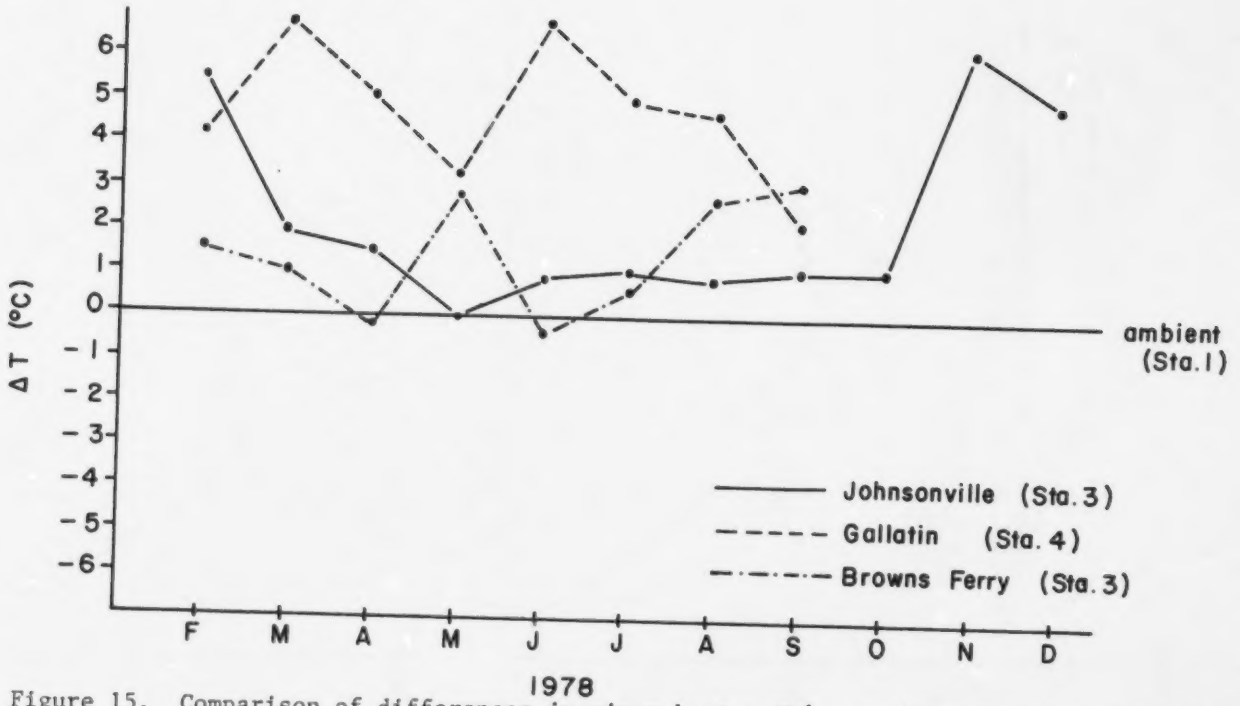


Figure 15. Comparison of differences in river bottom ΔT's at the thermal plume stations where H. bilineata nymphs were collected in the vicinity of Johnsonville and Gallatin Steam Plants and Browns Ferry Nuclear Plant.

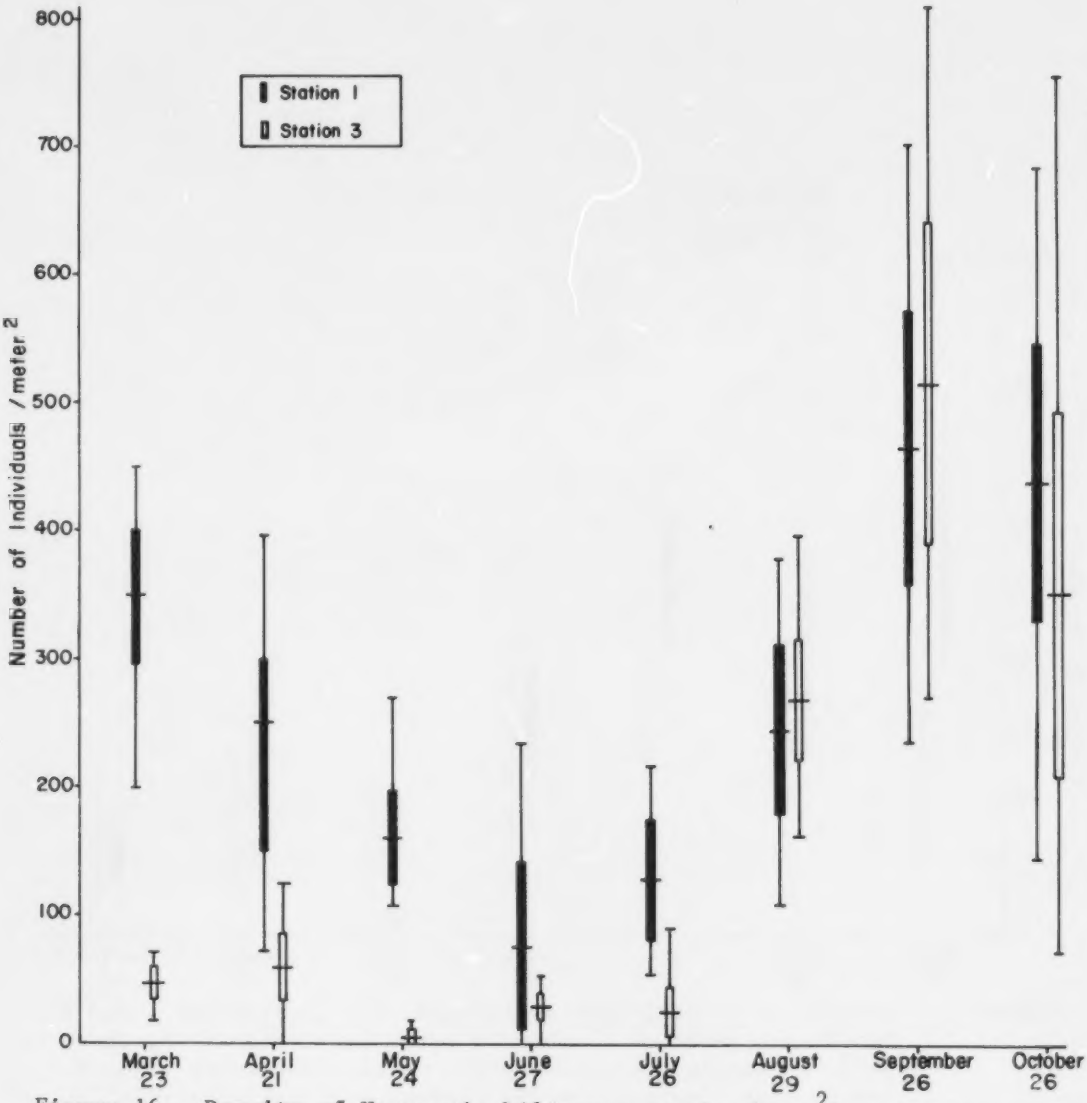


Figure 16. Density of Hexagenia bilineata nymphs (no.m^2) at stations 1 and 3 near Johnsonville Steam Plant from March to October 1978 based on 10 Ponar samples per station per month. Horizontal line = sample mean, rectangle = 95% confidence limits, vertical line = range.

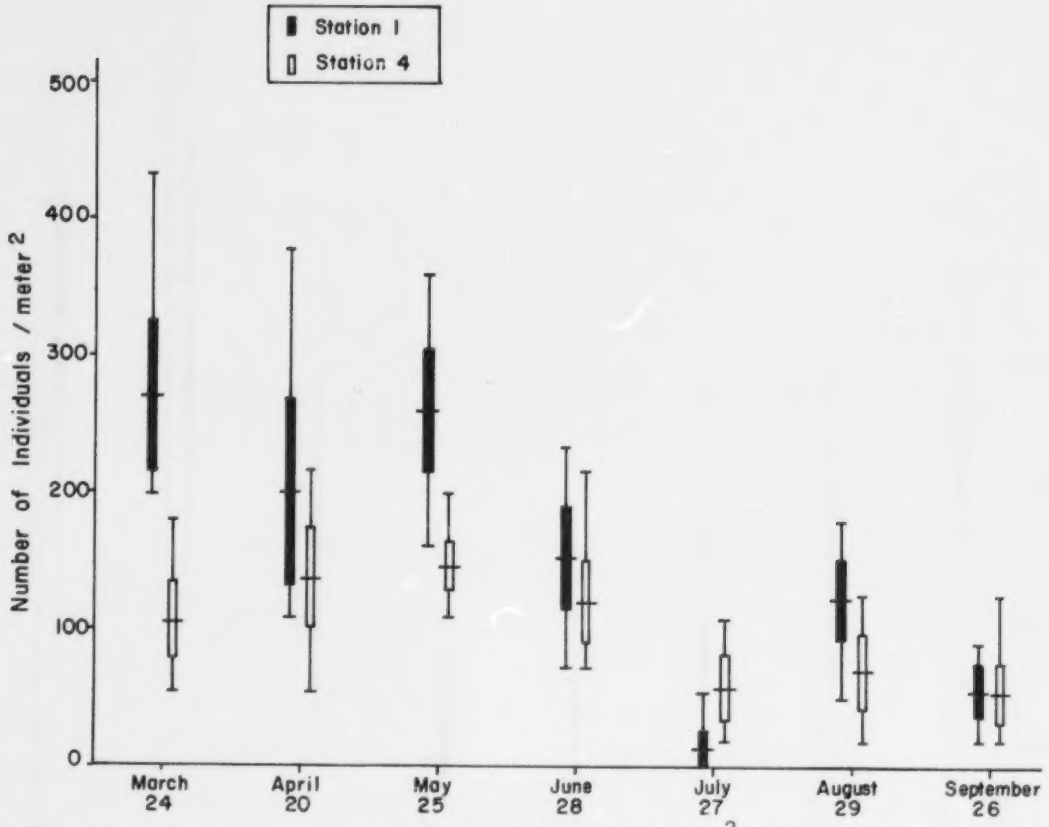


Figure 17. Density of H. bilineata nymphs (no. m^2) at stations 1 and 4 near Gallatin Steam Plant from March to October 1978; based on 10 Ponar samples per station per month. Horizontal line = sample mean, rectangle = 95% confidence limits, vertical line = range.

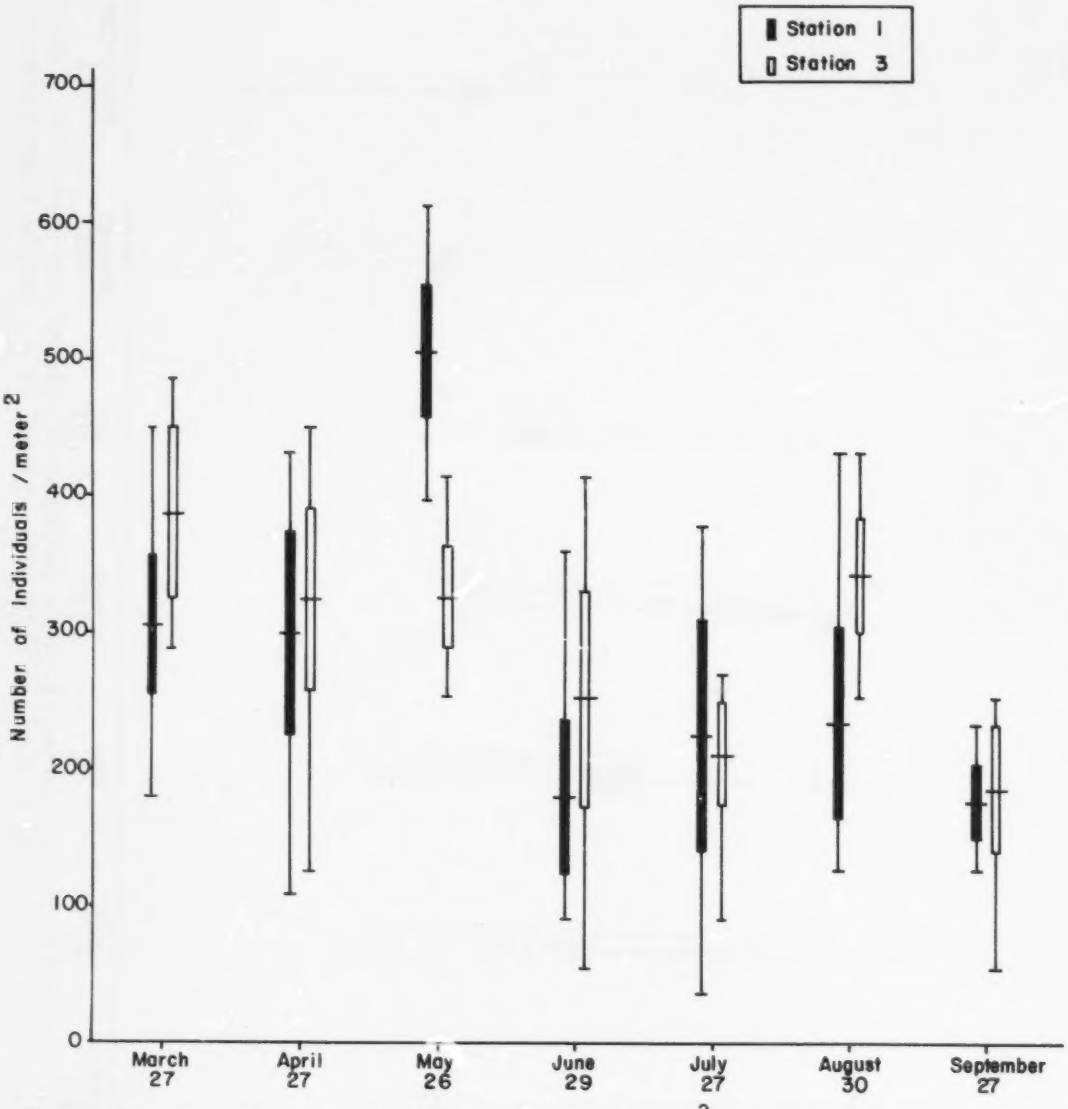


Figure 18. Density of H. bilineata nymphs (no./^{2}) at stations 1 and 3 near Browns Ferry Nuclear Plant from March to September 1978; based on 10 Ponar samples per station per month. Horizontal line = sample mean, rectangle = 95% confidence limits, vertical line = range.

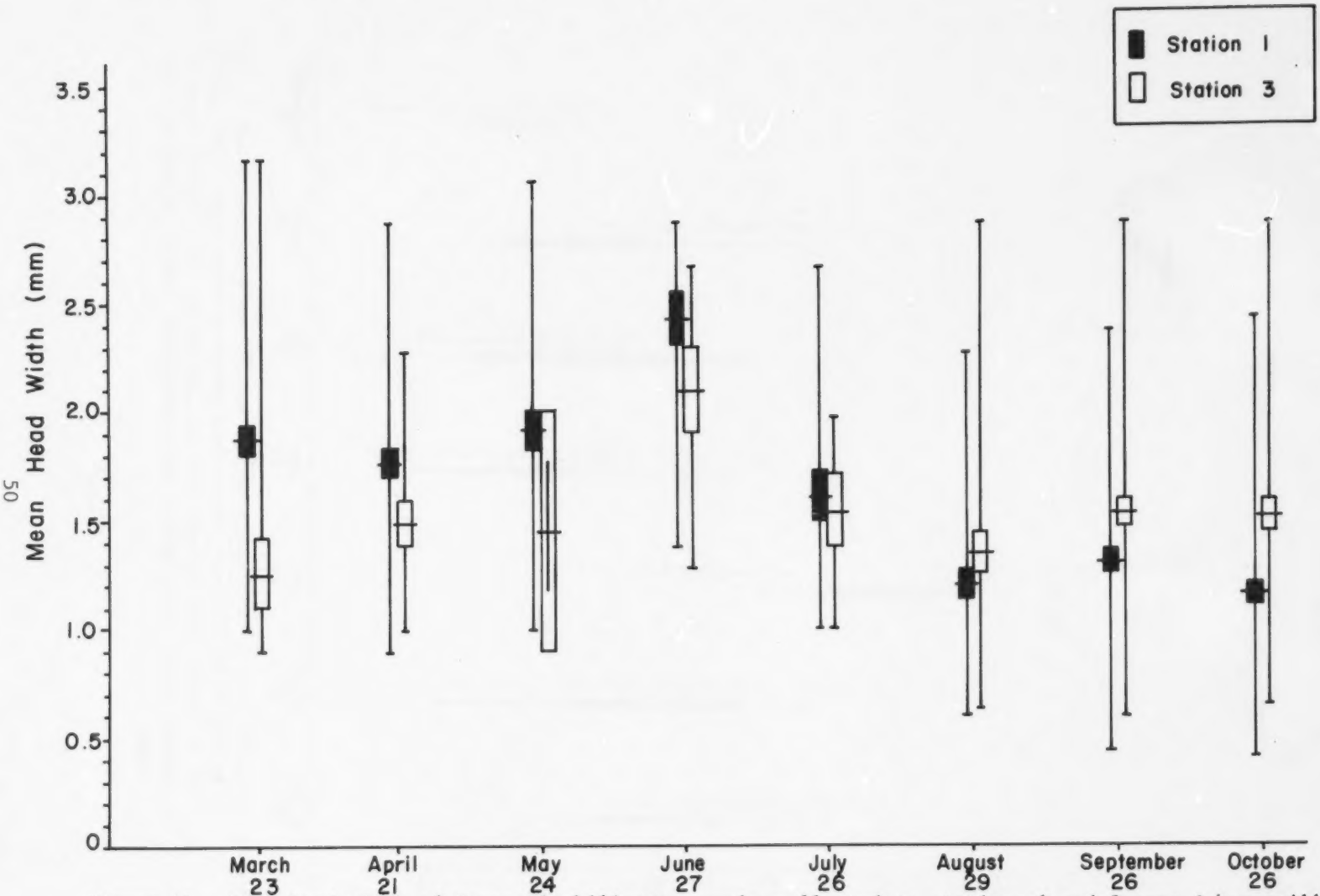


Figure 19. Mean head width of Hexagenia bilineata nymphs collected at stations 1 and 3 near Johnsonville Steam Plant from March to October 1978. Horizontal line = sample mean, rectangle = 95% confidence limits, vertical line = range.

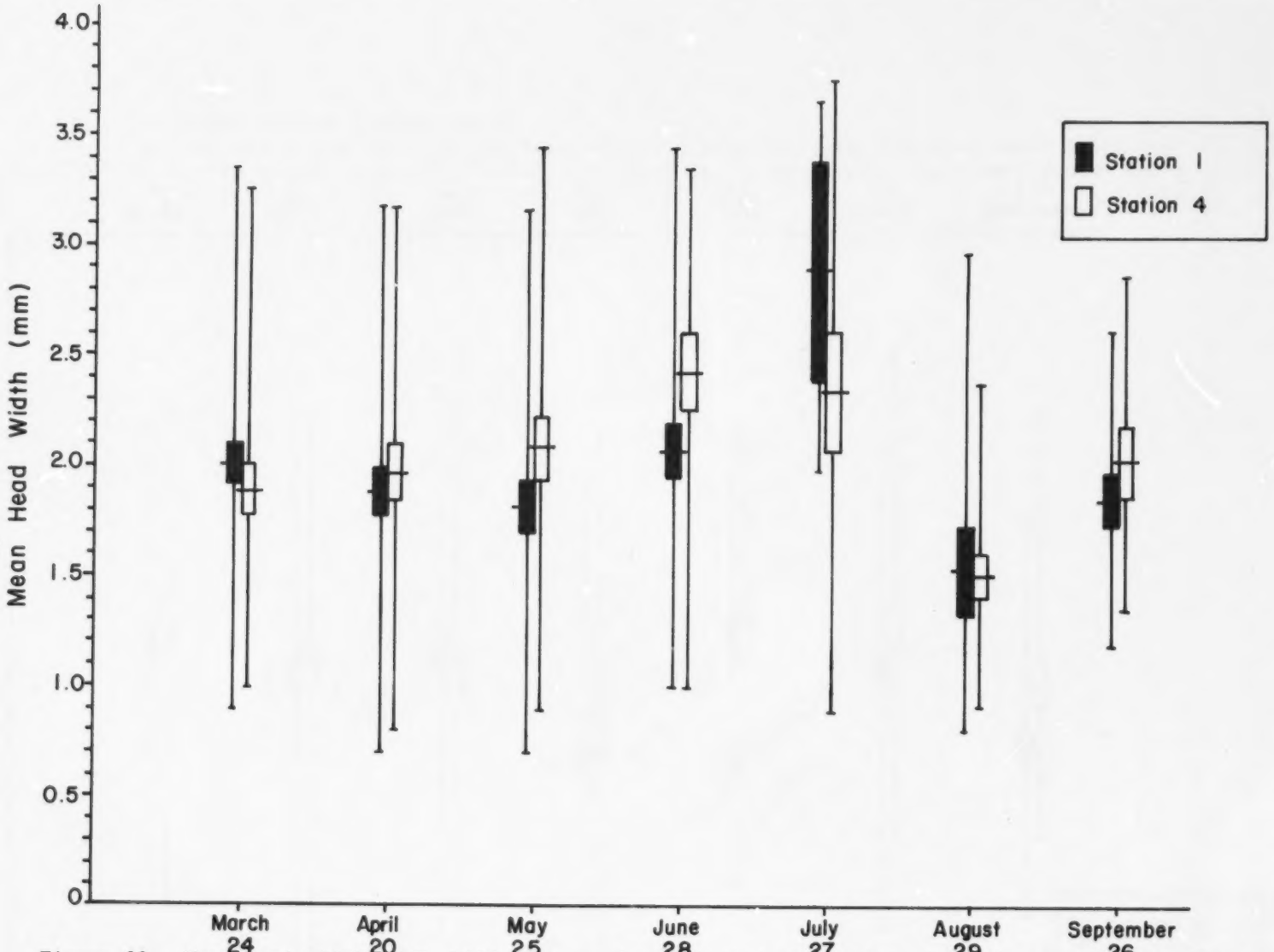


Figure 20. Mean head width of H. bilineata nymphs collected at stations 1 and 4 near Gallatin Steam Plant from March to September 1978. Horizontal line indicates mean, rectangle indicates 95% confidence limits.

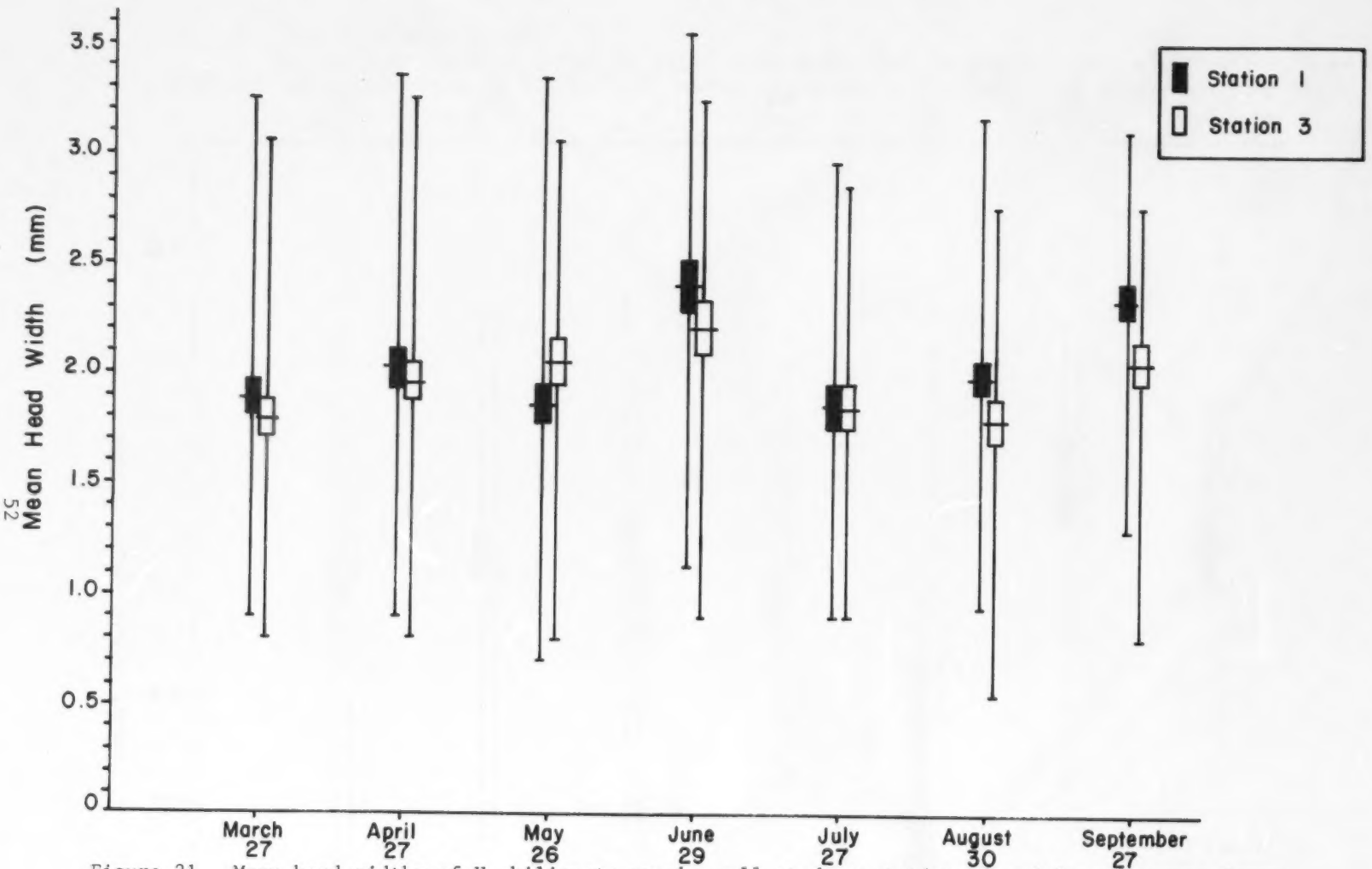


Figure 21. Mean head widths of H. bilineata nymphs collected at stations 1 and 3 near Browns Ferry Nuclear Plant from March to September 1978. Horizontal line indicates mean, rectangle indicates 95% confidence limits.

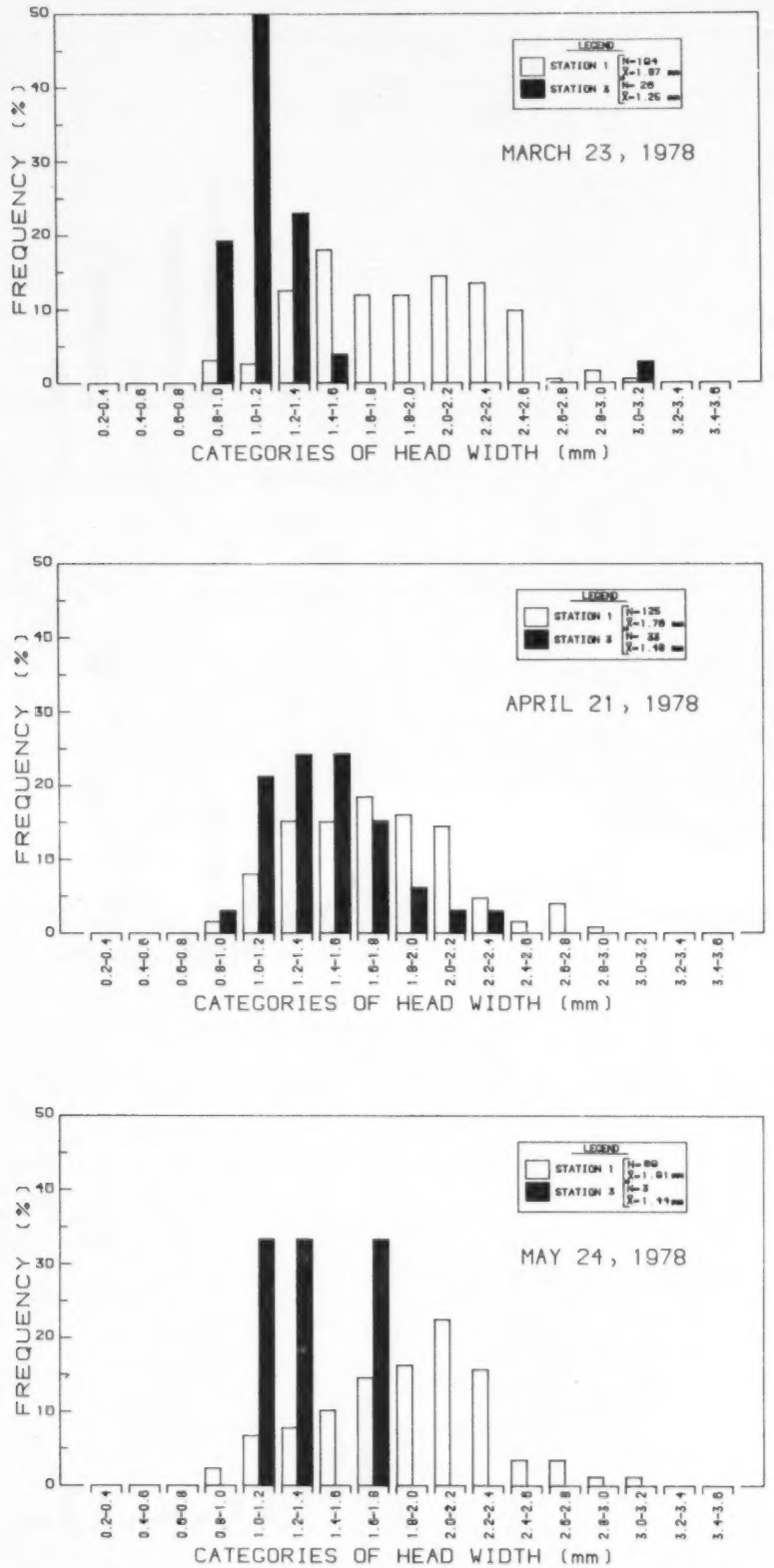
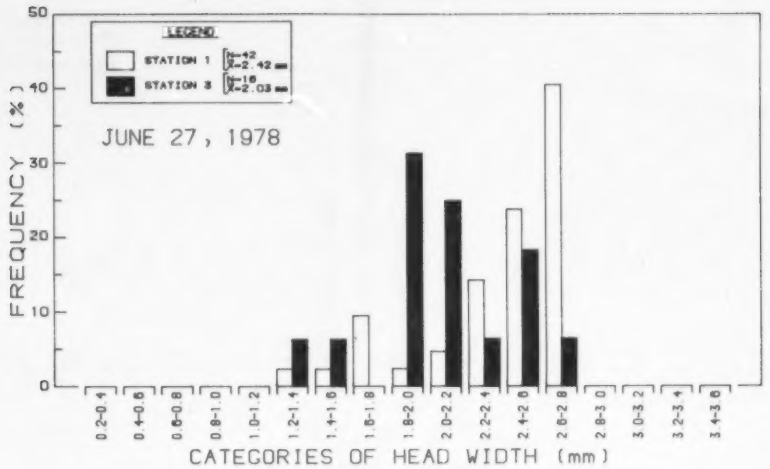
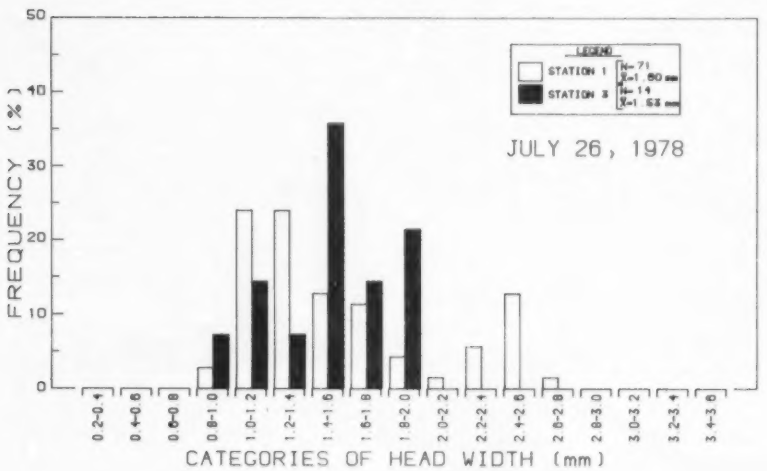


Figure 22. Comparisons of frequency (%) of Hexagenia bilineata nymphs in 0.2 mm size classes of head width at stations 1 (ambient) and 3 (thermal) near Johnsonville Steam Plant.





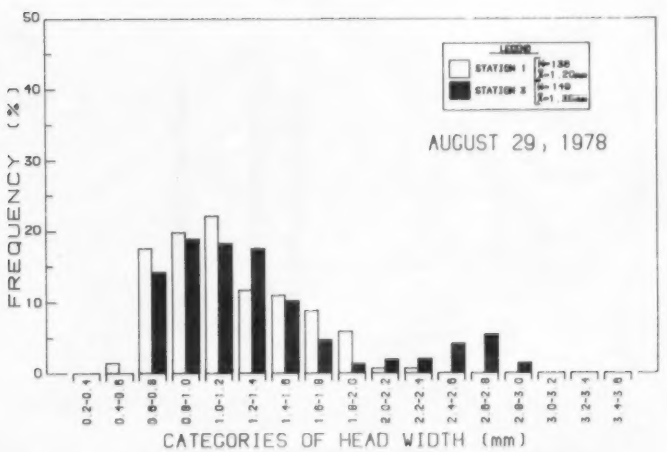
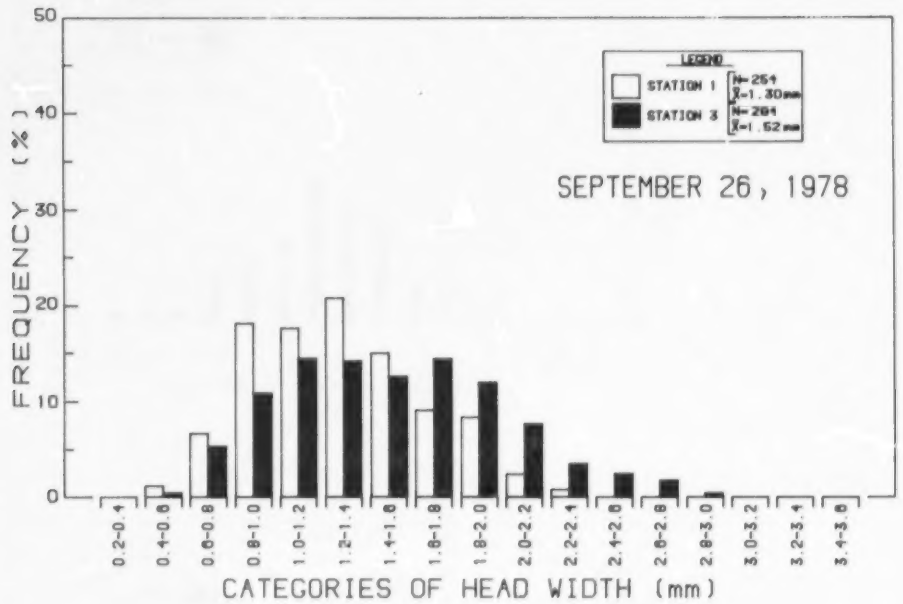


Figure 22.(continued).



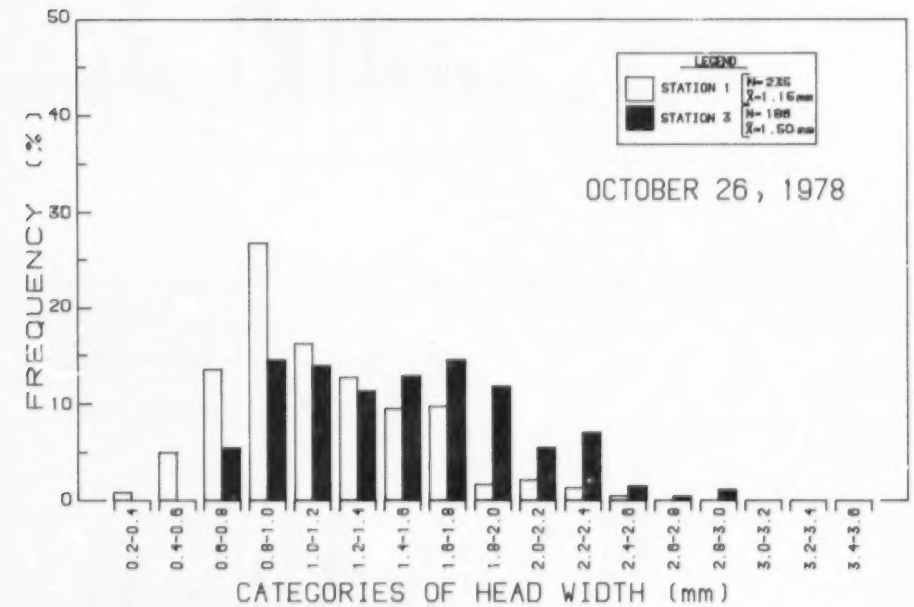


Figure 22. (continued).

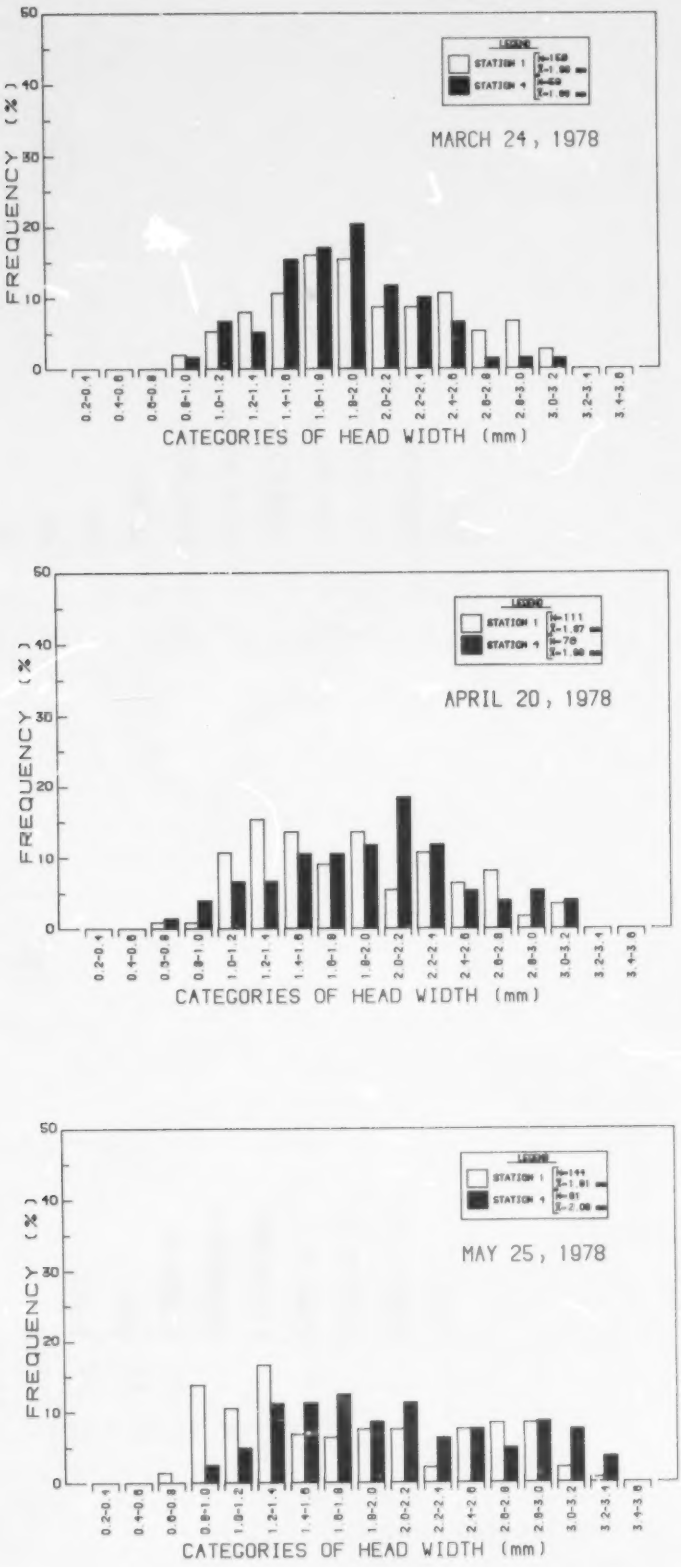
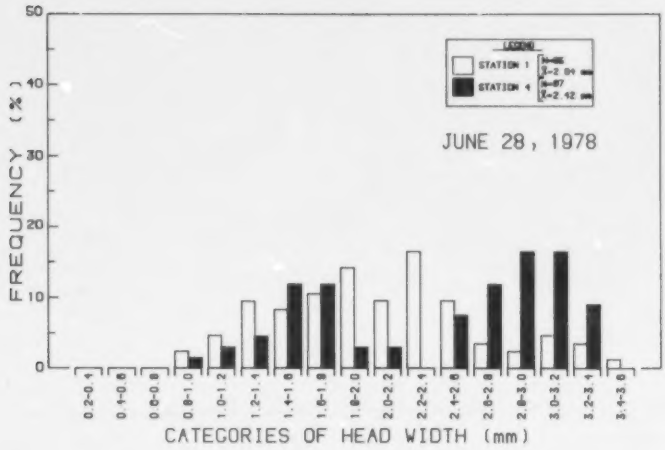
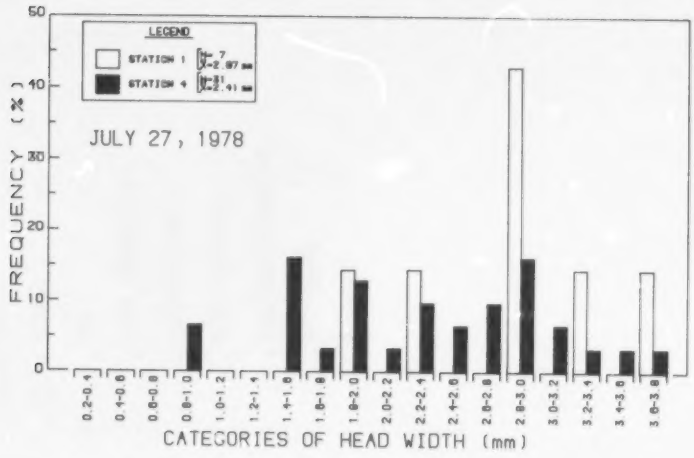


Figure 23. Comparisons of frequency (%) of Hexagenia bilineata nymphs in 0.2 mm size classes of head width at stations 1 (ambient) and 4 (thermal) near Gallatin Steam Plant.





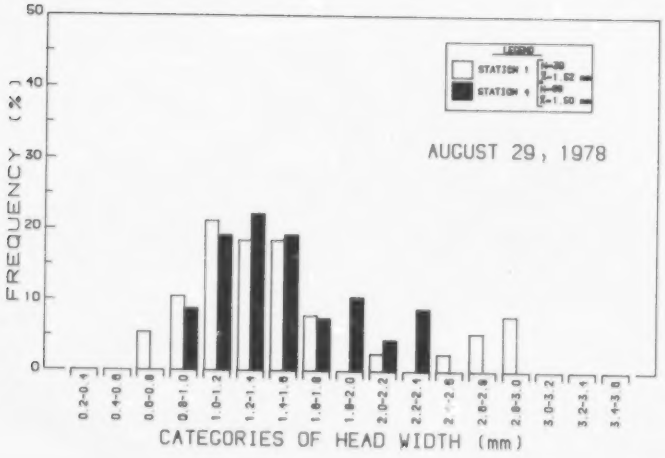


Figure 23. (continued).

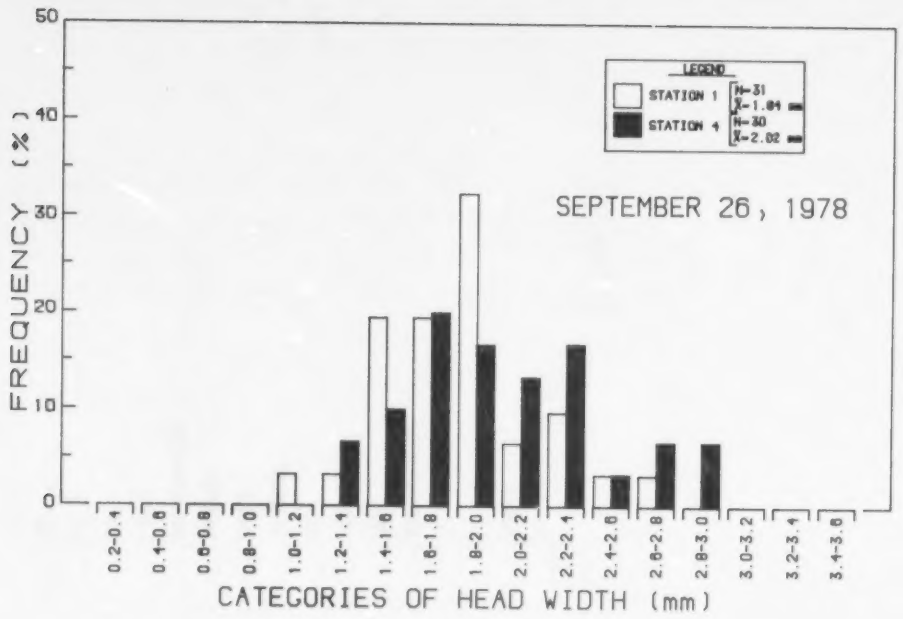
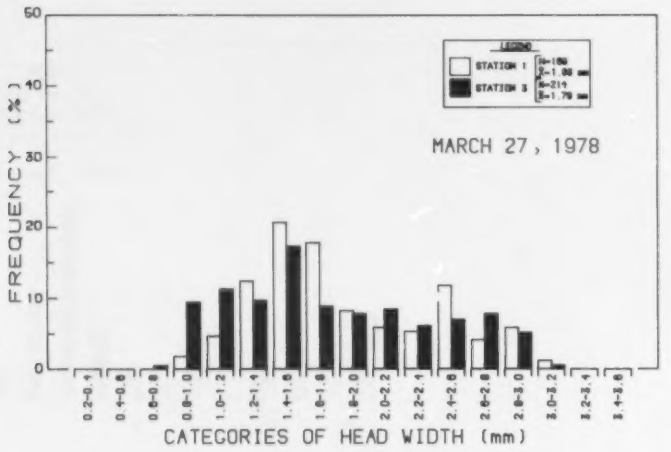
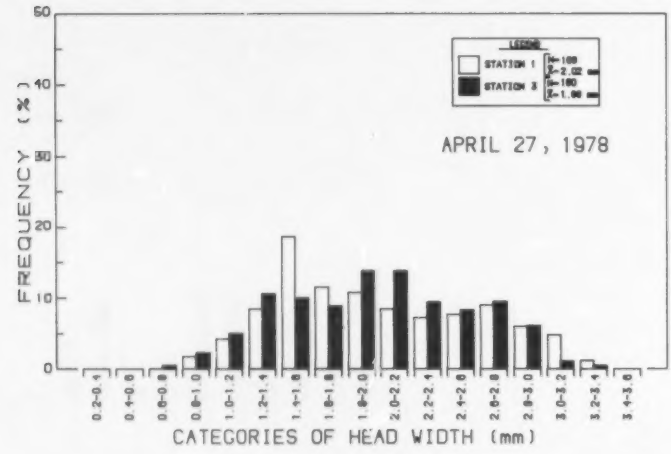


Figure 23. (continued).





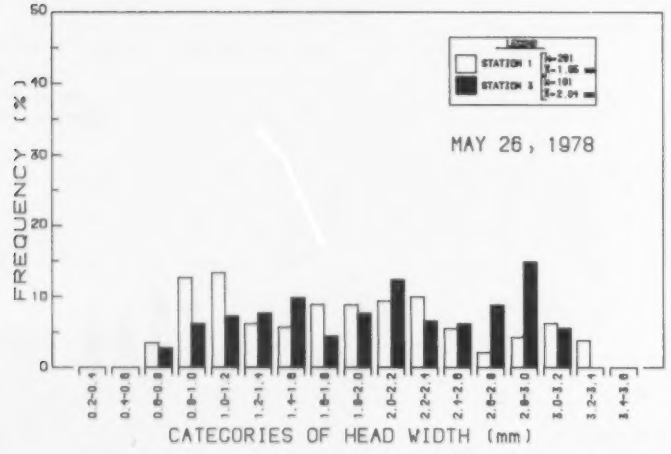
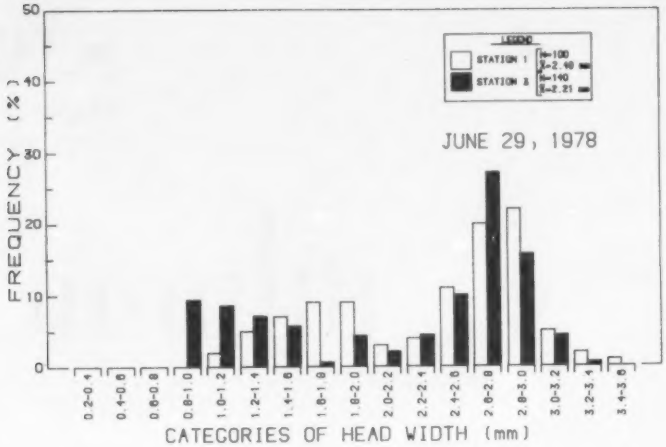
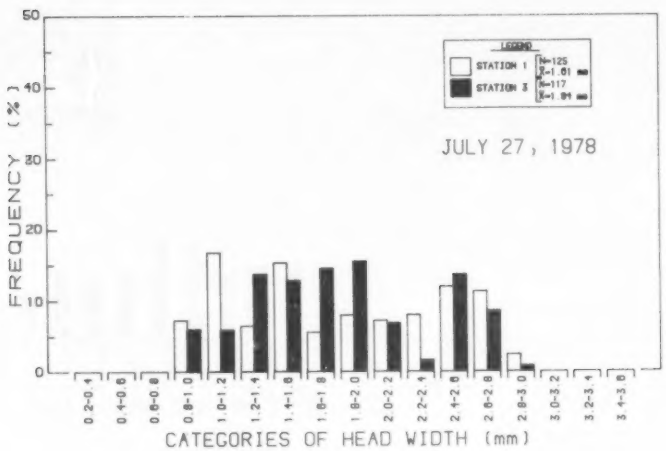


Figure 24. Comparisons of frequency (%) of Hexagenia bilineata nymphs in 0.2 mm size classes of head width at stations 1 (ambient) and 3 (thermal) near Browns Ferry Nuclear Plant.





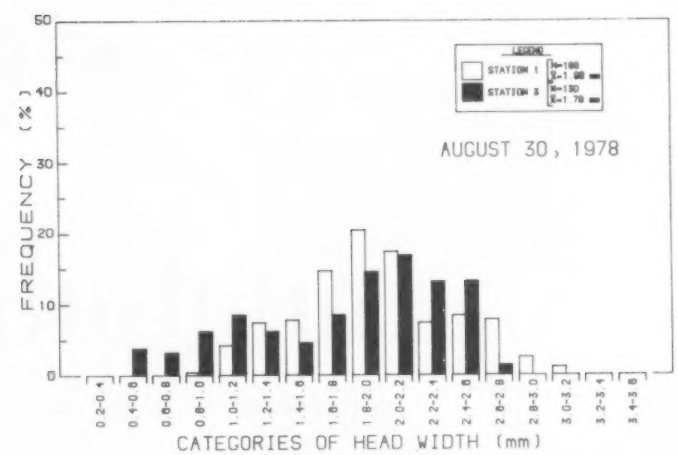


Figure 24.(continued).

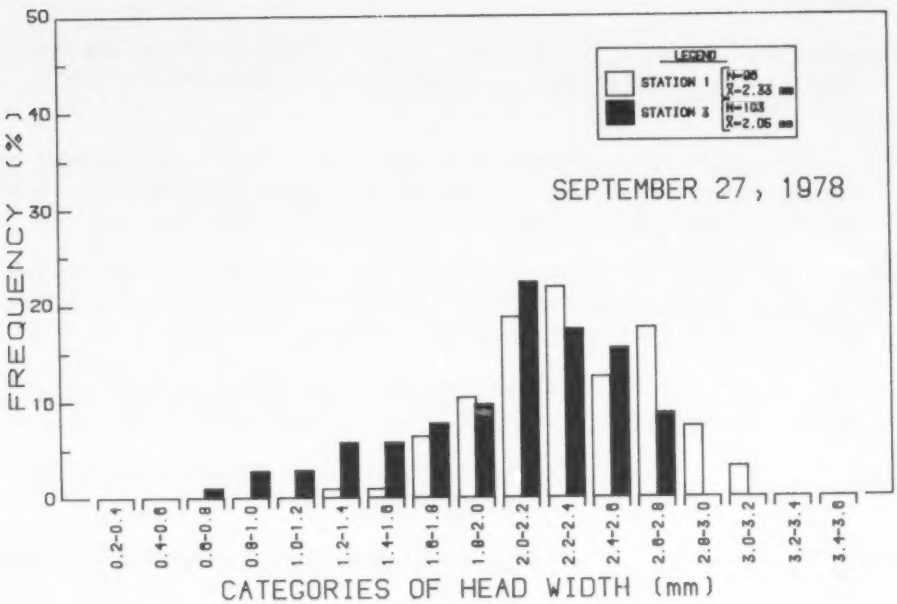


Figure 24. (continued).

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GLOSSARY

acclimation - adaptation to new conditions, usually requiring several days to one week.

acclimation temperature - temperature to which organism is adapted.

ambient - surrounding environmental condition.

diapause - a delay in the growth or development of an insect.

emergence - metamorphosis from the pupa or last-instar nymph into the adult insect.

eurythermal - occurring over a temperature range of 15°C or greater.

hemimetabolous - of simple metamorphosis, from egg to nymph (which resembles adult) to adult; wings develop externally.

holometabolous - of complex metamorphosis, from egg to larva to pupa to adult; wings develop internally.

incipient lethal temperature - highest temperature to which organism can be continuously exposed for an indefinite period without increasing the mortality rate.

instar - a stage in the life cycle of an insect between molts.

mesothermal - occurring at temperatures between 15 and 30°C; temperate-water forms.

poikilotherm - a cold-blooded organism.

thermal plume - warm water discharged from once-through cooling by electric generating plants; boundary is 2°C above ambient isotherms.

thermal shock - abrupt change in environmental temperature.

thermal tolerance - ability to survive extreme changes in temperature.